EXPERIENTIA

REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE

Editores:
P. HUBER · R. MATTHEY · A. v. MURALT · L. RUZICKA
Basel Lausanne Bern Zürich
Redactor: H. MISLIN, Basel-Mainz

BIRKHÄUSER VERLAG BASEL
SUISSE – SCHWEIZ – SVIZZERA – SWITZERLAND

UNIVERSITY OF HAWAII LIBRARY DEC 4 '59

SOMMAIRE – INHALT – SOMMARIO – CONTENTS

P.-P. Grassé et Ch. Noirot: L'évolution de la symbiose chez les Isoptères 365

Brèves communications - Kurze Mitteilungen - Brevi comunicazioni - Brief Reports

R. Hirt und R. Berchtold: Biophysikalische Studien mit synthetischem Lezithin 373
G. Krampitz und W. Albersmeyer: Über die Trennung von Phenol-Derivaten an Ionenaustauscherharzen . . 375
J. Tomcsik et Mélina Bouille: Effet cytologique des traitements mécanique et ultrasonique sur quelques bacilles aérobies 375
W. Lührs, G. Bacigalupo, B. Kadenbach und E. Heise: Der Einfluss von Chlorpromazin auf die oxydative Phosphorylierung von Tumormitochondrien 376
L. Å. Hanson and B. Johansson: Immune Electrophoretic Analysis of Bovine Milk and Purified Bovine Milk Protein Fractions 377
H. P. von Hahn: Catalase Activity in the Regenerating Tail Tip of Xenopus Larvae and the Effect of 3-Amino-1,2,4-triazole 379
M. Rybak, M. Petakova, V. Mansfeld, and J. Hladovec: Die Komplement-hemmende Wirkung einiger Anti-proteasen 380
Å. Bertler and E. Rosengren: On the Distribution in Brain of Monoamines and of Enzymes Responsible for their Formation 382
S. Garattini, A. Jori, V. Palma, and L. Tessari: On the Hypoglycemizing Effect of Metahexamide 384
Barbara Robert, L. Robert et M. F. Jayle: Les glycoprotéines sériques dans le granulome expérimental provoqué par la carrageenine 385
Joanne L. Gellerman and H. Schlenk: Column Chromatography of Lipides: Odd-Numbered Straight-Chain Fatty Acids of Menhaden Oil 387
V. Bolcato and G. Leggiero: Probable Mechanism of the Oxidation of Acetate to Glycolate by the Way of the Glycolaldehyde 388
I. Faredin, M. Winter, B. Tanos, and G. Hetényi: Catecholamines and Histamine in Vascular Tissue of Normal and Depancreatised Dogs 389
B. Gözsy and L. Kátó: Behavior of Capillary Endothelium During Dextran Induced Oedema 391
N. P. Buu-Hoi, N. D. Xuong et A. Beauvillain: Différence d'activité oestrogène entre l'α-bromotriphényl-éthylène et un de ses analogues pentadeutérié 392
B. Giovanella, C. Manni, and G. Moricca: Turbidimetric Detection of Decamethonium 393
E. Lüthy, B. Bischof und H. Rösli: Das Verhalten des Glykogens bei linksventrikulärer Hypertrophie nach artifizieller peripherer Widerstandserhöhung 394
Gy. Sávay and B. Csillik: Lead Reactive Substances in Peripheral Synapses 396
H. Gangloff und T. J. Haley: Veränderungen der elektrischen Hirntätigkeit bei Katzen nach Röntgentotalbestrahlung 397
J. F. W. Nuboer: Optically Aimed Ear Movements in the Rabbit 399

Informations - Informationen - Informazioni - Notes

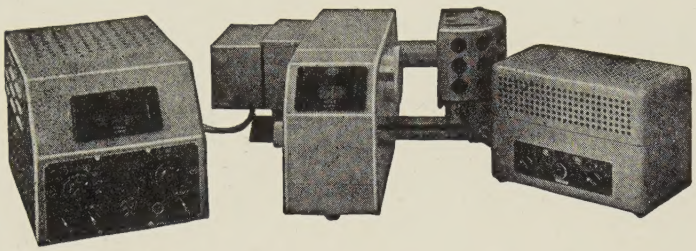
R. Stewart: Atom Transfer Mechanisms in Oxidation Processes (Studiorum Progressus) 401
Congressus 406
R. F. Vollman: Carl G. Hartman's Contributions to the Physiology of Reproduction (in memoriam) 407

ZEISS

Das Quarz-Spektralphotometer

PMQ II

erfüllt Ihre Wünsche!



Beachten Sie die Vorteile:

- **Direktanzeige** des Meßwertes (ohne Einbuße an Meßgenauigkeit), dadurch Übersicht über das Absorptionsspektrum und Zeitgewinn.
- **Vollständiger Netzbetrieb.** Keine Akkumulatorwartung; jederzeit betriebsbereit.
- **Impulslicht – Wechselstromverfahren.** Dunkelstromeinstellung fällt weg und ebenso der feuchtigkeitsempfindliche Gleichstromverstärker (keine Trocknungspatronen mehr).
- **Meßbereich:** 200–2500 mμ.
- **Höchste Empfindlichkeit** durch Sekundärelektronen-Vervielfacher 1P28 und PbS-Photo-Widerstand.
- **Einfach-Monochromator** mit Quarz- oder Glasoptik oder **Doppel-Monochromator** mit auswechselbarer Quarz- und Glasoptik nach Wunsch.
- **Mikroküvetten** mit nur 0,2 cm^3 Füllvolumen pro Zentimeter Schichtlänge.
- **Flammen-, Fluoreszenz-, Remissions- und Chromatogrammzusätze.**

Bitte verlangen Sie den ausführlichen Prospekt Nr. 50–657/E

Zuverlässige Beratung und Service durch eigenes Fachpersonal

CARL ZEISS **Generalvertretung für die Schweiz:**

GANZ *Optar* AG
ZÜRICH
Bahnhofstr. 40 Tel. (051) 25 16 75

Verzeichnis der Inserenten – Liste des annonceurs – List of Advertisers – Experientia XV/10

Atlas-Werke AG, Bremen
Ciba AG, Basel
Ganz Optar AG, Zürich
J. R. Geigy AG, Basel
Maggioni & C, S. p. A., Milano
Metrohm AG, Herisau
E. Mettler, Zürich
E. Renggli, Zug
Sandoz AG, Basel

Die EXPERIENTIA kann in folgenden Ländern durch die Post abonniert werden:
Des abonnements pour EXPERIENTIA peuvent être commandés près des bureaux de poste des pays suivants:

Italien	Belgien, Luxemburg	Dänemark	Schweden	Portugal
Deutschland	Holland	Norwegen	Finnland	Marokko

L'EXPERIENTIA paraît le 15 de chaque mois. Vente et abonnement dans toutes les librairies suisses et étrangères, ou directement chez l'éditeur. Abonnement pour un an frs. 68.–. Ce prix s'entend en francs suisses.

Die EXPERIENTIA erscheint am 15. jedes Monats und kann im In- und Ausland durch jede Buchhandlung oder direkt vom Verlag bezogen werden. Der Abonnementspreis beträgt Fr. 68.–.

Insertionspreise: $^1/_1$ Seite Fr. 220.–, $^1/_2$ Seite Fr. 132.–, $^1/_4$ Seite Fr. 77.–. Inseratenannahme durch den Verlag.

EXPERIENTIA esce al 15 di ogni mese e puo esser richiesta ad ogn libreria svizzera o estera, o anche direttamente alla casa editrice. L'abbonamento annuo è di fr. 68.–. Il prezzo vanno intesi in valuta svizzera.

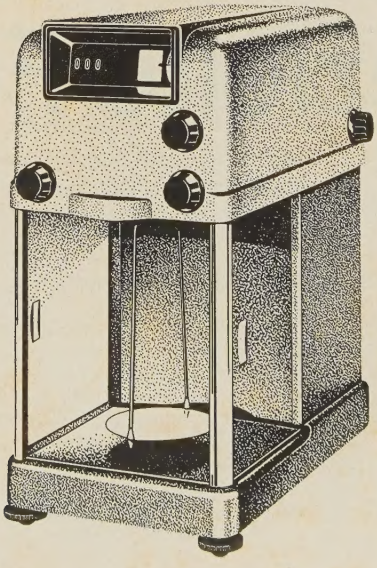
EXPERIENTIA is published on the 15th of every month and can be obtained in any country through the booksellers or from the publishers. The price by annual subscription is fr. 68.–. Price in Swisscurrency.

Prices for advertising: $^1/_1$ page fr. 220.–, $^1/_2$ page fr. 132.–, $^1/_4$ page fr. 77.–. Advertisements should be sent to the publishers.

© *1959 Birkhäuser Verlag, Basel 10 Schweiz, Elisabethenstrasse 19, Telephon (061) 23 18 10; Telegrammadresse: Edita Basel*

Printed in Switzerland by Birkhäuser AG., Basel

Pelikanstrasse 19
Zürich



Analysen-Waagen
Typ H
200 mg
optische
Skala

Am Anfang jedes chemischen Produktes steht die Forschung, und zwar

gezielte Forschung

Zufallstreffer sind selten geworden. Ein gut aufeinander abgestimmtes Räderwerk, in dem jeder Forscher Teilaufgaben löst, die näher und näher zum Ziel führen, bis eines Tages der Wurf gelingt, ist heute der einzig gangbare Weg. In den Geigy-Laboratorien führen Hunderte von qualifizierten Mitarbeitern jährlich Zehntausende von Versuchen durch. Dieser hohe Einsatz in gezielter Forschung bürgt dafür, daß Geigy auch in Zukunft bei Verbrauchern in der ganzen Welt auf Vertrauen zählen darf.

Geigy

J.R. Geigy A.G., Basel

A 7

EXPERIENTIA

Vol. XV - Fasc. 10 Pag. 365–408 15. X. 1959

L'évolution de la symbiose chez les Isoptères

Par P.-P. Grassé et Ch. Noirot*

La différenciation des ordres actuels au sein des Blattoptéroïdes est extrêmement ancienne; deux d'entre eux, les Dictyoptères (Blattes) et les Isoptères (Termites) ont dû s'isoler dès le Paléozoïque, peut-être même à la fin du Dévonien[1]. Au cours d'une très longue période, la *symbiose* a subi une évolution remarquable, dont nous pensons pouvoir retracer quelques étapes.

Très tôt, la symbiose s'est établie entre les Blattoptéroïdes et des microorganismes. La plus ancienne, intracellulaire, n'est pas liée aux processus digestifs. La deuxième, de beaucoup la plus intéressante, est liée à la xylophagie, ou plus exactement à l'utilisation de la cellulose. Le remarquable dans cette deuxième sorte de symbiose, c'est qu'elle fait appel, selon les groupes considérés, à des Protistes différents et à des mécanismes distincts.

I. *Symbiose non liée au régime alimentaire*

Les bactériocytes du tissu adipeux. Les Blattes hébergent, dans leur tissu adipeux, des microorganismes symbiotiques, localisés dans des cellules particulières (*bactériocytes*) et transmis à la descendance par infestation directe des ovocytes encore contenus dans l'ovaire maternel[2,3]. Il s'agit d'une véritable symbiose comme l'ont montré les expériences récentes de suppression des microorganismes[4]; les Blattes stérilisées sont fragiles, dépigmentées, et surtout leur fécondité est extrêmement faible; elles souffrent vraisemblablement de l'absence de quelque substance normalement synthétisée par leurs symbiotes. De tels symbiotes ont été observés dans de nombreuses espèces de Blattes; en fait, dans toutes celles où on les a cherchés; il est très probable que cette association est générale dans les espèces actuelles. Aussi est-il très significatif d'observer le *même* type d'association chez le Termite *Mastotermes darwiniensis*[5] avec le *même* mode de transmission[6]. Ce Termite est le seul représentant actuel de la famille très primitive des Mastotermitidae; il est donc logique de penser que ce type de symbiose est *antérieur* à la séparation des deux lignées, et devait déjà exister chez l'ancêtre des Isoptères, vers la fin de l'Ere primaire.

II. *Symbiose liée à la xylophagie*

A. *Les Flagellés.* La symbiose avec des Protozoaires Flagellés, vivant dans une dilatation de l'intestin postérieur, est étroitement liée au régime xylophage. Les Flagellés, qui ont subi une évolution complexe, une diversification remarquable[7], sont les véritables auteurs de la digestion de la cellulose, peut-être grâce à des Bactéries intracellulaires, si bien qu'on aurait une symbiose au 2e degré, mais cette hypothèse, due à Pierantoni[8] ne repose que sur l'observation de microorganismes, dont certains sont constants (cas de *Joenia annectens*) au sein du cytoplasme de certains Flagellés. Privés de leurs symbiotes, les Termites ne peuvent survivre longtemps[9,10] et Hungate[11] a minutieusement analysé les processus fermentaires anaérobies par lesquels les Flagellés dégradent la cellulose en CO_2, H_2 et acide acétique. Les Protozoaires sont généralement éliminés au cours de la mue, et sont réacquis par trophallaxie[12]; l'un de nous a donné une revue complète de ces problèmes[13].

Chez les Termites, ces Flagellés sont présents de façon constante dans *toutes* les espèces primitives (familles des Mastotermitidae, Calotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae) et sont au contraire absents chez les Termites supérieurs (famille des Termitidae), bien que beaucoup de ceux-ci aient conservé le régime xylophage. Il est très remarquable de constater que la même association existe aussi chez la Blatte *Cryptocercus punctulatus*, de la famille des Panesthiidae, xylophage, dont la faune de Flagellés, très variée, présente des affinités très étroites avec celle des Termites, deux genres (*Oxymonas* et *Trichonympha*) étant même communs[14]. *Cryptocercus* étant à coup sûr une Blatte très primitive, il est permis de penser que

* Laboratoire d'Evolution des Etres Organisés, Paris.

[1] A. V. Martynov, Trav. Inst. Paleont. Acad. Sci. U.R.S.S. *7*, 150 (1938).

[2] A. Koch, Mikrokosmos *38*, 6 (1949).

[3] P. Buchner, *Endosymbiose der Tiere mit pflanzlichen Mikroorganismen* (Birkhäuser, Bâle 1953, 771 p.

[4] A. G. Richards et M. A. Brooks, Ann. Rev. Entomol. *3*, 37 (1958).

[5] C. Jucci, Arch. Zool. ital. *16*, 1422 (1932); *Nature 169*, 837 (1952).

[6] A. Koch, Zool. Anzeig. Suppl. *11*, 81 (1938).

[7] P.-P. Grassé, *Traité de Zoologie, Anatomie Systématique, Biologie*, vol. *I* (1952).

[8] D. U. Pierantoni, Boll. Soc. ital. Biol. sper. *10*, 944 (1935).

[9] B. Grassi et A. Foa, R. C. Accad. Lincei *20*, 725 (1911).

[10] L. R. Cleveland, Proc. nat. Acad. Sci., Wash. *9*, 424 (1923); Biol. Bull. *46*, 203 (1924).

[11] R. E. Hungate, Ecology *19*, 1 (1938); Ecology *20*, 230 (1939); Ann. ent. Soc. Amer. *36*, 730 (1943).

[12] P.-P. Grassé et Ch. Noirot, Bull. biol. Fr. Belg. *79*, 273 (1945).

[13] P.-P. Grassé, Tijdsch. Entomol *95*, 70 (1952).

[14] L. R. Cleveland, S. R. Hall, E. P. Sanders et J. Collier, Mem. Amer. Acad. Arts Sci. *17*, 185 (1934).

l'association avec des Flagellés est très ancienne; l'ancêtre commun des Blattes et des Termites possédait sans doute déjà le régime xylophage, et devait présenter un *double symbiose:* avec des microorganismes intracellulaires d'une part, avec des Flagellés intestinaux d'autre part. Dans la faune actuelle, deux espèces ont conservé cette double symbiose ancestrale: *Mastotermes darwiniensis* chez les Termites, *Cryptocercus punctulatus* chez les Blattes.

Les Flagellés symbiotiques manquent chez les autres Blattes étudiées jusqu'ici; la présence de Flagellés complexes, à fouets nombreux n'est cependant pas obligatoirement liée, chez les Blattoidea, à la xylophagie. Les *Lophomonas* de *Blatta, Blatella, Periplaneta,* toutes Blattes cosmopolites et omnivores, en fournissent la preuve. Mais le rôle de ces Flagellés dans la digestion de la cellulose n'est pas connu et les *Lophomonas* manquent très souvent dans l'intestin postérieur des Blattes; en outre, ces Flagellés se transmettent d'un individu à un autre par des kystes, la contamination d'un nouvel hôte est tout à fait aléatoire[15]; il ne s'agit sûrement pas d'une symbiose. L'intérêt des *Lophomonas* est de montrer que le saprophytisme simple peut avoir sur des Entozoaires une action morphogénétique (indirecte bien sûr) extrêmement puissante. Inversement, le régime xylophage a persisté malgré la perte des Flagellés cellulolytiques; chez les *Panesthia,* la digestion du bois s'effectue grâce à des Bactéries situées dans l'intestin *antérieur*[14] et peut-être des Amibes (voir p. 282). Chez les Termites supérieurs, qui forment la grande famille des Termitidae, la symbiose paraît revêtir des aspects variés et complexes.

B. – *La symbiose dans la famille des Termitidae.* Chez les Termitidae, *plusieurs* sortes de symbiose ont apparu. D'une part, une flore bactérienne spécialisée prospère dans le tube digestif, probablement chez tous les Termitidae, et nous pressentons l'existence de plusieurs types de telles associations; d'autre part, le phylum des Macrotermitinae a acquis en outre un type de symbiose tout à fait différent, avec un Champignon qui végète sur les amas de bois mâché, ou meules, que les ouvriers construisent dans leur nid; enfin, on observe chez certaines espèces des Protistes intestinaux (Amibes, Ciliés) localisés dans un segment donné du tube digestif et dont la présence paraît constante, mais le caractère symbiotique de telles associations est encore purement hypothétique. Examinons ces divers cas.

1° *Les Bactéries intestinales.* Le tube digestif présente chez tous les Termites un développement considérable; son anatomie est très uniforme dans les familles inférieures, mais il a subi, chez les Termitidae, une évolution complexe dont nous avons commencé l'étude[16–18]. Notons seulement ici les deux points les plus importants. Tout d'abord, le cloisonnement de l'intestin postérieur: chez les Termites inférieurs, ce segment présente une seule dilatation, ou *panse,* dont l'entrée est commandée par la *valvule entérique,* située au début de l'intestin postérieur. Parmi les Termitidae, cette disposition est conservée chez les Macrotermitinae et beaucoup de Nasutitermitinae. Dans les autres cas, l'intestin postérieur est plus complexe, et présente plusieurs dilatations successives plus ou moins bien individualisées; le plus souvent, la valvule entérique est déplacée vers l'arrière et sépare les deux premières «panses» (Termitinae, la plupart des Amitermitinae), mais parfois aussi cette valvule garde sa position antérieure (Apicotermitinae, Nasutitermitinae). Dans tous les cas, elle débouche dans une vaste «panse». En second lieu, la jonction de l'intestin moyen et de l'intestin postérieur se présente souvent de façon inhabituelle, si bien qu'il existe un *segment mixte* (*S. M.*, Fig. 2 et 3) en arrière du mésentéron proprement dit, où le tube digestif est limité sur une de ses faces par une paroi d'intestin moyen, sur l'autre par l'épithélium proctodéal avec son intima chitineuse (Termitinae, la plupart des Amiterminae et des Nasutitermitinae).

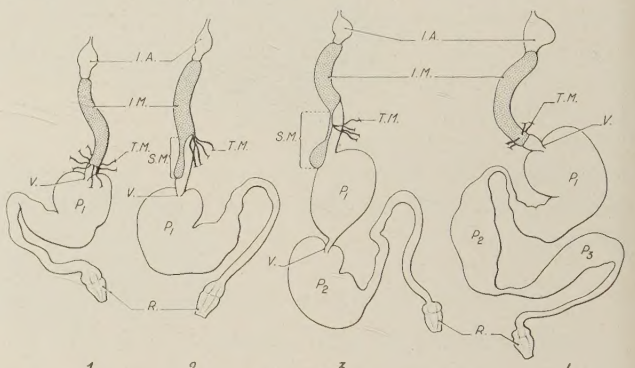


Fig. 1 à 4. Tube digestif de quelques Termites. *1* forme primitive (*Calotermes flavicollis*); *2, 3, 4,* quelques exemples de Termitidae: *2 Trinervitermes* (Nasutitermitinae); *3 Amitermes* (Amitermitinae); *4 Jugositermes* (Apicotermitinae). *I. A.* intestin antérieur; *I. M.* intestin moyen; P_1, P_2, P_3, dilatations («panses») de l'intestin postérieur; *R.* rectum; *S. M.* segment mixte; *T. M.* tubes de Malpighi; *V.* valvule entérique.

Les Figures 1 à 4 représentent quelques exemples de cette différenciation du tube digestif chez les Termidae, par comparaison avec une forme primitive (*Calotermes*).

L'intérêt de l'étude anatomique réside dans le fait que la flore intestinale, pour une espèce de Termites donnée, varie considérablement suivant le segment considéré, et que, à l'examen microscopique, la composition et la densité de cette flore paraissent sensiblement constante d'un individu à un autre pour un segment déterminé.

Il n'est ni dans nos intentions ni de notre compétence d'entreprendre une étude détaillée de ces flores bactériennes; nous voulons simplement attirer l'attention sur quelques aspects de ces associations, sur leur localisation, leur complexité, d'après l'étude histologique d'un grand nombre d'espèces.

[15] P.-P. Grassé, Arch. Zool. expér. génér. *65,* 6 (1926).
[16] P.-P. Grassé et Ch. Noirot, Ann. Sci. nat. Zool. *16,* 345 (1954).
[17] Ch. Noirot et J. Kovoor, Insectes Soc. *5,* 439 (1958).
[18] J. Kovoor, Bull. Soc. zool. Fr., sous presse.

L'intestin antérieur, quoique parfois très volumineux (notamment chez les mangeurs d'humus), paraît dépourvu de microorganismes, il en est généralement de même de l'intestin moyen, sauf dans les cas exceptionnels mentionnés plus loin. Dans les régions suivantes, on observe au contraire, dans toutes les espèces de Termites, des associations bactériennes d'apparence très variée, dont nous pouvons distinguer 3 types d'après leurs rapports topographiques avec la masse alimentaire: microorganismes libres, extérieurs au bol alimentaire; microorganismes fixés aux parois, mais en contact avec les aliments (flore «pariétale»); microorganismes libres mélangés au contenu intestinal.

Fig. 5. *Termes hospes:* Coupe du tube digestif au niveau du segment mixte. On voit l'amas de Bactéries (*B*) localisé entre le bol alimentaire (*A*) et la paroi, uniquement sur la face où celle-ci est formée par l'intestin moyen (*I. M.*), alors que les bactéries sont absentes en regard de l'épithélium proctodéal (*I. P.*). (× 400)

a) Dans les espèces où le tube digestif présente un *segment mixte*, on observe au niveau de celui-ci des Bactéries particulières, dont la localisation est bien précise (Fig. 5). Le plus souvent, les microorganismes sont situés entre le bol alimentaire et la paroi, mais uniquement du côté du *mésentéron* (*Termes, Pericapritermes, Nasutitermes, Microcerotermes, Globitermes, Amitermes, Eremotermes*). Les Bactéries dont la densité est très grande (Fig. 5) présentent l'aspect d'une culture pure; suivant les espèces de Termites, leur forme est très variée (depuis de courts bâtonnets jusqu'à de longs filaments flexeux), mais pour un Termite donné on ne trouve généralement à ce niveau qu'*une seule forme* bactérienne, parfois deux (*Microcerotermes*[18]); l'amas bactérien ne se mélange pas au contenu intestinal; celui-ci d'ailleurs est souvent encore entouré de la membrane péritrophique, et en outre, les replis de l'épithélium proctodéal, qui bordent la jonction des deux tissus et font saillie dans la cavité, tendent parfois à subdiviser celle-ci, si bien que la partie endodermique du segment mixte forme une poche à l'écart du transit intestinal; ce cas, fréquent chez les Amitermitinae (*Microcerotermes, Eremotermes*), est celui où la localisation des Bactéries du segment mixte est la plus stricte. Dans les autres, où la cavité n'est pas ou presque pas subdivisée, les Bactéries pénètrent, en arrière, dans l'intestin postérieur proprement dit, mais toujours sur une très faible longueur. Dans d'autres cas, les Bactéries du segment mixte se placent *tout autour* du bol alimentaire, et non plus seulement vers le mésentéron (*Thoracotermes, Noditermes, Cubitermes, Trinervitermes*), mais ne se mélangent en aucune manière aux aliments. Enfin, dans les genres tels que *Syntermes, Cornitermes, Promirotermes*, où le segment mixte est très dilaté, les Bactéries se localisent bien du côté mésentérique, mais leur séparation d'avec le bol alimentaire est moins absolue.

De telles flores bactériennes paraissent très générales chez les Termitidae pourvus d'un segment mixte; la seule exception que nous ayons rencontrée est *Cephalotermes rectangularis*, où le segment mixte, bien différencié, ne nous a montré aucun microorganisme, aussi bien dans des specimens provenant de Côte d'Ivoire, que dans d'autres récoltés au Moyen-Congo.

Chez les Termites où il n'existe pas de segment mixte (jonction mésentéron-proctodéum normale), on ne peut évidemment pas observer des faits strictement identiques. Pourtant, dans quelques cas, on observe, au voisinage de cette jonction, une flore bactérienne comparable aux précédentes puisqu'elle se tient entre la paroi intestinale et le bol alimentaire (le plus souvent encore enfermé dans la péritrophique); ces Bactéries peuvent être observées dans la partie tout à fait postérieure de l'intestin moyen (*Coxotermes, Heimitermes*), ou bien dans la partie tout à fait antérieure du proctodéum (*Ceratotermes, Eutermellus*).

En revanche, on n'observe rien d'analogue à ce niveau du tube digestif chez les Macrotermitinae, chez la plupart des Apicotermitinae, chez *Mimeutermes*.

Sur la signification physiologique de telles associations microbiennes nous ne pouvons que faire des hypothèses; le caractère symbiotique de telles associations nous paraît très probable, étant donné leur généralité, leur constance, leur localisation bien précise, mais seules des expériences permettront d'être affirmatifs. Il est peu vraisemblable que ces Bactéries interviennent dans la *digestion* des aliments; d'une part, elles restent extérieures à la masse alimentaire, d'autre part elles sont situées dans une région où le transit intestinal est rapide. En revanche, il importe de souligner leurs rapports avec les tubes de Malpighi; tout d'abord, cette

flore bactérienne pullule dans la région où les tubes de Malpighi débouchent dans l'intestin; ensuite, les tubes de Malpighi s'appliquent contre la partie mésentérique du segment mixte et y forment extérieurement un peloton serré, suggérant l'existence à ce niveau d'échanges d'un type particulier[17,18]; et c'est précisément à ce niveau (mais intérieurement) que prolifèrent les Bactéries. Chez les Termites où il n'y a pas de segment mixte, on constate des faits analogues; chez *Coxotermes* et *Heimitermes* (Apicotermitinae), on observe des Bactéries dans la partie postérieure de l'intestin moyen, or dans cette sous-famille les tubes de Malpighi débouchent *dans* le mésentéron[16]. Chez *Ceratotermes*, les Bactéries se voient au début de l'intestin postérieur, là où l'épithélium est très mince et où les tubes de Malpighi l'entourent d'un lacis serré[17].

b) En règle générale *on n'observe aucun développement bactérien dans la partie antérieure du proctodeum*, plus précisément dans la portion située en avant de la valvule entérique. Chez les Nasutitermitinae, les Apicotermitinae, les Macrotermitinae, cette première partie est très courte, mais chez les Amitermitinae et les Termitinae, elle est beaucoup plus développée, le plus souvent dilatée en une «panse» volumineuse. Quoiqu'il en soit, le bol alimentaire montre dans cette région le même aspect microscopique que dans l'intestin moyen, on n'y voit pratiquement aucun microorganisme ou du moins ne forment-ils jamais d'amas visibles; quand on observe des populations bactériennes à ce niveau, il s'agit soit d'une extension vers l'arrière des Bactéries du segment mixte (et jamais elles ne s'éloignent beaucoup de la limite postérieure de ce segment), soit de cas analogues précédemment décrits (*Ceratotermes*), c'est-à-dire de Bactéries nettement séparées du bol alimentaire. Ainsi, *avant la valvule entérique, on ne constate aucune prolifération bactérienne au sein du bol alimentaire*; parfois cependant, on observe quelques amas bactériens près de la paroi, ou même fixés à elle (*Termes, Cubitermes, Cornitermes*), mais ces amas sont de peu d'importance tant par leur nombre que par leurs dimensions. *Amitermes* fait exception à cette règle; le segment mixte communique très largement avec la première dilatation de l'intestin postérieur, et cette dilatation héberge, surtout dans sa partie antérieure, une grande quantité de microorganismes qui se mélangent aux débris alimentaires. Dans tous les autres cas, ou bien on n'observe pratiquement aucun développement microbien avant la valvule entérique (Macrotermitinae, la plupart des Apicotermitinae), ou bien il s'agit de microorganismes qui pullulent à l'extérieur de la masse alimentaire, sans y pénétrer (Bactéries le segment mixte ou cas analogues), et en restent séparées par la membrane péritrophique.

Le tableau change complètement dès que l'on franchit la valvule entérique. Dans tous les cas, cette valvule s'ouvre dans une vaste dilatation de l'intestin postérieur, où elle forme un entonnoir d'invagination; autour de l'ouverture de la valvule, il y a une pullulation extraordinaire de microorganismes, généralement de plusieurs types. Dans bien des cas la densité bactérienne est telle que les microbes forment *la majeure partie* du contenu intestinal à ce niveau, et le contraste est frappant avec la lumière de la valvule elle-même, dont le contenu paraît à peu près abiotique (Fig. 6); dans d'autres genres (*Rostrotermes, Allognathotermes, Mimeutermes*), la densité de la flore est moins grande, mais on observe malgré tout une augmentation considérable des Bactéries. Cette densité microbienne diminue quand on s'éloigne de l'ouverture de la valvule entérique, mais elle reste notable, de très nombreux microorganismes, de type varié, se mélangent aux débris alimentaires.

Fig. 6. *Pericapritermes chiasognathus:* coupe au niveau de la valvule entérique (*V*) qui sépare la première (P_1) et la deuxième (P_2) dilatation de l'intestin postérieur. Noter le changement brusque dans l'aspect du contenu intestinal à l'orifice de la valvule; dans la partie antérieure de la deuxième «panse» P_2, les microorganismes forment l'essentiel du contenu intestinal, et lui donnent, sur la photographie, un aspect très dense et une texture très fine. (× 175)

Outre cette flore abondante et variée, mélangée aux aliments, on observe par endroits une densité bactérienne considérable *au contact de la paroi intestinale* (Fig. 7); là encore, il y a une grande variété de microorganismes; les formes bacillaires et filamenteuses sont le plus souvent orientées perpendiculairement à la paroi et y paraissent même fixées. Mais le foisonnement microbien est en contact direct avec les débris alimentaires et s'y mélange intimement. Cette flore, que nous appellerons *flore pariétale*, n'est jamais répartie de façon uniforme. En général, elle est très développée

au voisinage de la valvule entérique, diminue vite et beaucoup vers l'arrière et présente un nouveau développement, encore plus considérable, dans la région postérieure de la panse. Mais dans le détail, cette flore pariétale varie passablement.

Fig. 7. *Termes hospes:* partie postérieure de la chambre à fermentation, coupe montrant la *flore pariétale* fixée à la paroi mais en contact direct avec les aliments. (× 1000)

Après cette dilatation qui existe chez *tous* les Termites (la «panse rectale» des Termites inférieurs en est l'homologue), le reste de l'intestin postérieur a un développement extrêmement variable; assez court et simple chez les xylophages stricts, il est beaucoup plus long et plus complexe chez les mangeurs d'humus. D'un genre à l'autre, la microflore varie également beaucoup, avec en général un développement notable de la flore «pariétale», les Bactéries dispersées au sein des débris alimentaires étant toujours nombreuses, mais sans former de rassemblements particulier.

Quel peut être le rôle des associations bactériennes que nous venons de décrire? Sont-elles véritablement symbiotiques, ou ne s'agit-il que de commensaux, voire de parasites? Depuis longtemps, on pense que les Termitidae digèrent la cellulose grâce à des bactéries symbiotiques, et quelques expériences viennent appuyer cette hypothèse: Hungate[19] a isolé un *Actinomyces* cellulolytique d'*Amitermes minimus*; tout récemment, Pochon, de Barjac et Roche[20] ont mis en évidence, chez *Sphaerotermes sphaerothorax*, une flore bactérienne étonnamment comparable à celle de la panse des Mammifères ruminants; d'autre part, Misra et Ranganathan[21] montrent que la cellulase, chez *Odontotermes obesus*, n'est pas sécrétée par les Termites, mais est probablement d'origine bactérienne.

L'examen histologique nous indique que cette cellulolyse doit se produire surtout dans le dilatation qui suit la valvule entérique, et qui mérite le nom de *chambre à fermentation*. Cet examen nous suggère en outre que les processus fermentaires sont complexes, et font intervenir plusieurs espèces bactériennes (ce qui est en accord avec les recherches de Pochon, de Barjac et Roche[20]). Il pourrait y avoir une chaîne, une cascade de réactions, car le contenu de la chambre à fermentation paraît «hétérogène», chaque région ayant un aspect particulier non seulement par sa microflore, mais aussi, dans bien des cas, par un certain tri des débris alimentaires; les fragments les plus gros se trouvent au centre, la périphérie est occupée seulement par de très fins débris. Il semble que les aliments stagnent longtemps dans cette chambre à fermentation, dont le péristaltisme est peu intense (la musculature est très peu développée eu égard au volume du tube digestif à ce niveau), que le brassage est très lent, qu'ainsi *plusieurs* types de fermentation peuvent s'exercer *successivement* sur le matériel ingéré. Il est vraisemblable que les fermentations se poursuivent dans les segments suivants de l'intestin postérieur, au moins chez les mangeurs d'humus.

2° *Les Protozoaires.* *a*) Outre des Schizophytes variés, l'intestin des Termitidae héberge souvent des Protozoaires, dont certains sont sûrement des parasites (Grégarines de l'intestin moyen), d'autres des commensaux tolérés mais inutiles. Dans cette dernière catégorie, il faut ranger les petits Flagellés (Trichomonadides) signalés par Kirby[22] chez *Amitermes* et *Termes*, par Henderson[23] chez *Cubitermes*; les Infusoires du genre *Nyctotherus* sont sans doute également de ce type; ils ont été trouvés[22] chez *Amitermes sp.pl.* Nous avons nous-même observé des Ciliés appartenant au même genre chez la plupart des Apicotermitinae (*Jugositermes, Rostrotermes, Hoplognathotermes, Allognathotermes, Heimitermes, Coxotermes*), mais aussi chez *Eutermellus bipartitus* et chez *Bellicositermes bellicosus*. Ces Ciliés ne sont jamais très abondants, et se localisent à des niveaux variés de l'intestin postérieur.

b) Les *Amibes* participent sans doute à des associations plus intéressantes, peut-être véritablement symbiotiques. Kirby[22] a décrit plusieurs espèces appartenant aux genres *Endamoeba* et *Endolimax* qu'il a trouvées chez des Termites des genres *Amitermes* et

[19] R. E. Hungate, J. Bact. *51*, 51 (1946).

[20] J. Pochon, H. de Barjac et A. Roche, Ann. Inst. Pasteur *96*, 352 (1959).

[21] J. N. Misra et V. Ranganathan, Proc. Indian Acad. Sci. [B] *39*, 100 (1954).

[22] H. Kirby, Quart. J. micr. Sci. *71*, 189 (1927); Parasitology *24*, 289 (1932).

Termes (*Mirotermes*); Henderson[23] trouve 4 espèces d'Amibes (des genres *Endamoeba* et *Endolimax*) chez un *Cubitermes* du Kenya; Kirby[24] signale encore la présence de ces Amibes chez *Capritermes capricornis* de Madagascar et chez plusieurs espèces d'*Amitermes* d'Afrique du Sud et de l'Est; enfin, de Mello[25] observe des Amibes dans le genre *Cornitermes*.

Toutes ces Amibes ne jouent sans doute pas le même rôle à l'égard du Termite qui les héberge. Les unes en effet sont essentiellement bactériophages, les autres phagocytent en très grande quantité des éléments du bol alimentaire de l'Insecte. *Endamoeba beaumonti* trouvée par Kirby[22] dans cinq espèces d'*Amitermes* appartient au premier type, ainsi que *E. pellucida* et *Endolimax suggrandis* de *Cubitermes sp.*[23]; il est douteux que de tels Protistes aient un rôle symbiotique. En revanche, les Amibes qui absorbent en abondance les fragments des végétaux ingérés par le Termite jouent peut-être un rôle dans la digestion de ces éléments. Les Amibes de ce type paraissent extrêmement fréquentes dans la sous-famille des Termitinae, comme Kirby[24] l'a déjà noté; cet auteur signale des *Endamoeba* de ce type chez plusieurs espèces de *Termes* et chez *Capritermes capricornis;* Henderson[23] en décrit deux espèces chez *Cubitermes sp.* du Kenya; nous en avons nous-même observé chez *Termes hospes, Thoracotermes brevinotus, Apilitermes longiceps, Crenetermes albotarsalis, Noditermes curvatus, Cubitermes severus* (Fig. 8), *Megagnathotermes notandus, Ophiotermes grandilabius,* tous de la sous-famille des Termitinae, et chez *Mimeutermes giffardi* (Nasutitermitinae). De tous ces Termites, seul le genre *Termes* est xylophage, mais se nourrit de bois très altéré; les autres sont tous des mangeurs d'*humus*. Les Amibes se localisent essentiellement dans la dilatation qui suit la valvule entérique (chambre à fermentation) et y sont très abondantes. Ni la nature des substances que les Termites digèrent dans l'humus, ni les diastases qui interviennent ne sont connues. Il n'est pas interdit de penser que les Amibes jouent un rôle utile dans cette nutrition, à côté de la riche flore microbienne. Notons enfin que l'*Endamoeba javanica* intervient peut-être dans la digestion du bois chez la Blatte xylophage *Panesthia javanica*[26].

c) Les Infusoires Péritriches du genre *Termitophrya*[27] posent aussi un problème à la fois phylogénétique et physiologique. Nous les avons trouvés d'une part dans trois genres d'Apicotermitinae étroitement apparentés (*Jugositermes, Rostrotermes, Trichotermes*) et d'autre part chez *Pericapritermes* (s. f. des Termitinae). Dans les trois premiers genres, ils se trouvent dans une dilatation de l'intestin postérieur située avant le rectum (P_3, Fig. 4) où ils sont *très abondants*; chez *Pericapritermes* ils se localisent dans le *rectum* où ils n'abondent jamais et où leur quantité varie fortement d'un individu à un autre.

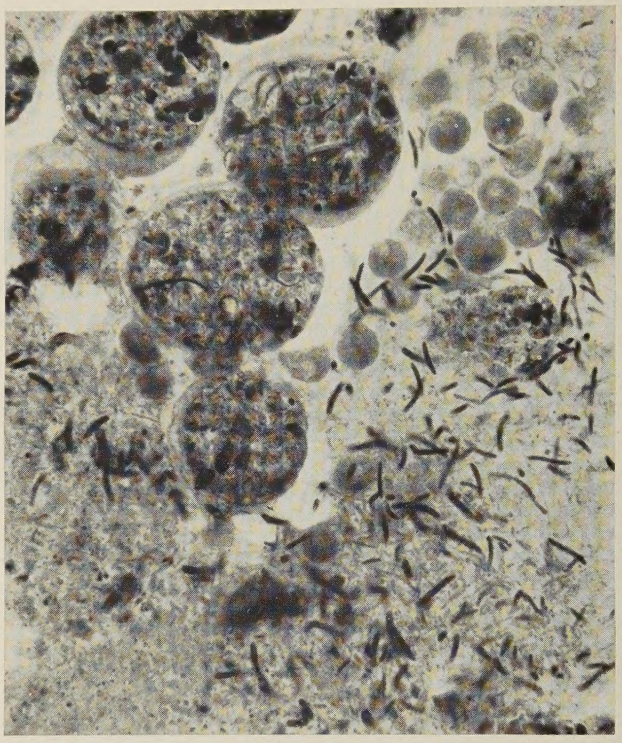


Fig. 8. *Cubitermes severus*. Aspect du contenu intestinal dans la chambre à fermentation, non loin du débouché de la valvule entérique. Outre de nombreuses Bactéries de taille et de forme variées, on observe deux types d'Amibes, les unes petites, à cytoplasme dépourvu d'ingestats, les autres, de grande taille, à membrane nucléaire épaisse (genre *Endamoeba*) bourrées d'enclaves alimentaires. (× 400)

Ces Infusoires sont des mangeurs d'humus, qu'ils ingèrent par leur oesophage spiralé, et dont leur endoplasme est littéralement bourré. Il serait prématuré de leur attribuer un rôle symbiotique, mais chez *Jugositermes, Trichotermes* et *Rostrotermes*, leur constance et leur extraordinaire abondance dans un segment bien déterminé de l'intestin postérieur, ne laissent pas d'être impressionnantes, et traduisent au moins une adaptation très marquée de ces Ciliés aux hôtes qui les hébergent. L'adaptation est-elle univoque ou réciproque (c'est-à-dire symbiotique)? L'expérience nous l'apprendra.

3° *Les Meules à champignons des Macrotermitinae.* La sous-famille des Macrotermitinae, qui constitue un phylum bien individualisé, a acquis un type de symbiose tout à fait nouveau. Un trait de comportement caractérise cette sous-famille: l'édification dans le nid d'amas de matière végétable broyée et imbibée de salive, sur lesquels végètent des Champignons du genre *Termitomyces*. (Dans la meule, on trouve aussi du mycélium de *Xylaria* qui, dans les conditions normales, ne se développe que fort peu et ne *fructifie pas*.) L'association Termite-Champignon est le fait d'une étroite spécialisation mutuelle, car l'architecture de la meule

[23] J. C. Henderson, Univ. Calif. Publ. Zool. *43*, 357 (1941).
[24] H. Kirby, Univ. Calif. Publ. Zool. *41*, 189 (1937).
[25] F. de Mello, S. Paulo Dept. Zool. Papeis Avulsos *11*, 345 (1954).
[26] G. W. Kidder, Parasitology *29*, 163 (1937).
[27] Ch. Noirot et C. Noirot-Timothée, C. R. Acad. Sci. sous presse (1959).

est caractéristique de l'espèce de Termite et les *Termitomyces* s'observent uniquement dans ce milieu bien particulier; les espèces de *Termitomyces* diffèrent d'ailleurs d'un genre de Macrotermitinae à un autre. La physiologie de cette association a donné lieu à bien des controverses, mais nous avons montré[28] que la meule à champignons est en perpétuelle transformation: les ouvriers apportent sans cesse de nouveaux matériaux et rongent sans cesse les parties anciennes; ils consomment ainsi la matière même de la meule après que celle-ci ait subi l'action des champignons; il y aurait une sorte de pré-digestion, dont nous cherchons actuellement à préciser la nature. Il y a sûrement attaque de la lignine, et de ce fait démasquage des matériaux utilisables (cellulose, hémicelluloses); mais ces derniers eux-mêmes subissent des modifications. Il est possible, en outre, que le champignon lui-même apporte aux Termites des facteurs de croissance[29–31].

III. *Symbiose et phylogénie*

Les associations symbiotiques et parasitaires présentent un intérêt majeur pour le Biologiste, car leur évolution implique un accord entre les modifications des deux partenaires. Cet accord est particulièrement évident dans le cas de la symbiose, où il y a adaptation *réciproque* des espèces associées; ici la corrélation est à double sens, alors qu'elle est à sens unique dans le cas du parasitisme. Nous avons tenté de suivre, chez les Blattoptéroïdes et spécialement chez les Isoptères, cette phylogénie parallèle des Insectes et des Symbiontes, et nous résumons cette tentative dans la Figure 9. Il est à peine besoin de souligner la grande part d'hypothèse qu'implique l'établissement d'un tel arbre généalogique; il ne s'agit pas dans notre esprit de proposer des conclusions définitives, mais plus simplement de présenter des hypothèses de travail, points de départ de recherches futures. La récente monographie de Seevers[32] nous montre à propos des Staphylins termitophiles l'intérêt de telles tentatives.

L'ancêtre Blattoptéroïde paléozoïque possédait, sans doute, une double symbiose: d'une part avec des Flagellés intestinaux, d'autre part avec des microorganismes vivant dans certaines cellules (bactériocytes); nous supposons en outre qu'il avait un régime *xylophage*. Les Mantodea ont bientôt perdu leurs symbiotes peut-être en liaison avec l'adoption d'un régime *carnivore;* les Blattodea ont abandonné en majorité la xylophagie, et corrélativement ont perdu leurs Flagellés; les Panesthiidae font exception, mais alors que *Cryptocercus* a gardé une riche faune de Flagellés, *Panesthia* a remplacé ceux-ci par des Bactéries cellulolytiques qui semblent jouer le même rôle physiologique, aidées peut-être par des Amibes. Les microorganismes des bactériocytes sont conservés chez toutes les Blattes,

[28] P.-P. Grassé et Ch. Noirot, C. R. Acad. Sci., Paris *244*, 1845 (1957); Ann. Sci. nat. Zool. *20*, 113 (1958).
[29] P.-P. Grassé, Ann. Sci. nat. Zool. *6*, 97 (1944); *7*, 115 (1945).
[30] Ch. Noirot, Ann. Sci. nat. Zool. *14*, 405 (1952).
[31] W. A. Sands, Insectes Soc. *3*, 531 (1956).
[32] C. H. Seevers, Fieldiana, Zool. *40*, 334 (1957).

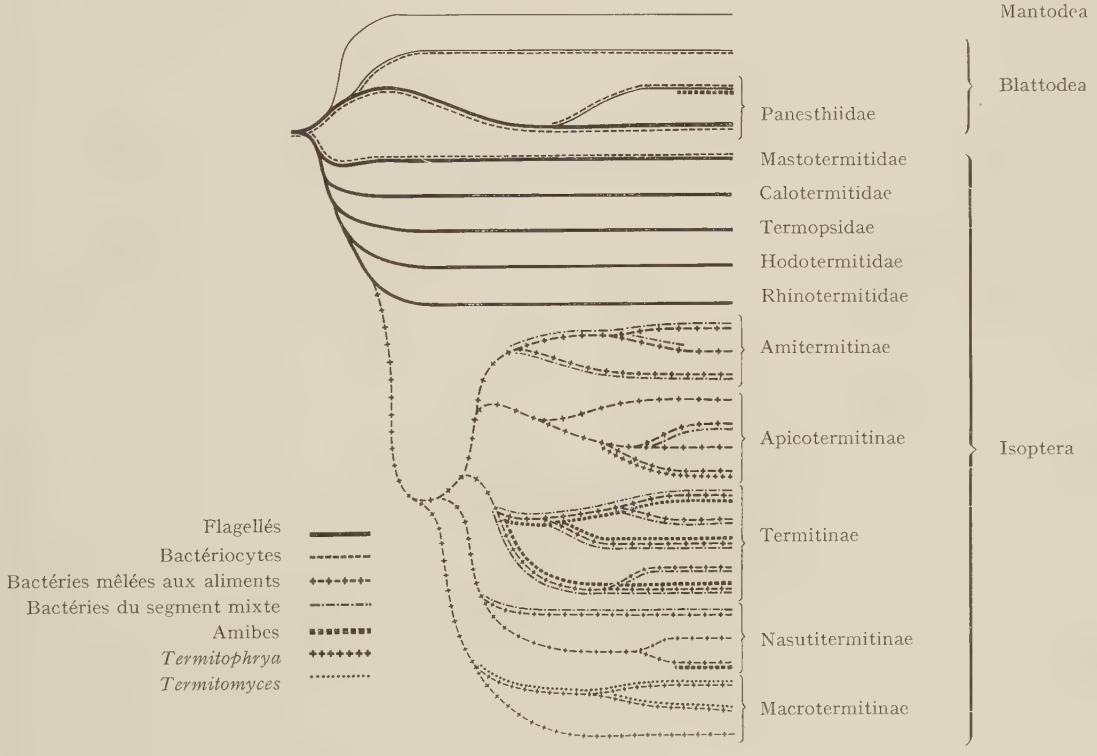


Fig. 9. Arbre généalogique des Blattoptéroïdes montrant l'évolution de la symbiose. (Explications dans le texte.)

mais n'ont persisté chez les Termites que dans d'archaïque *Mastotermes*, dont l'anatomie nous indique qu'il s'est isolé très tôt du tronc originel des Isoptères. Après la perte des bactériocytes, l'Evolution des Termites s'est poursuivie pendant longtemps sans modification notable des rapports symbiotiques, car les représentants des Calotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae sont des xylophages qui digèrent le bois grâce à leurs Flagellés. La diversification tant de ces Termites que de leurs Flagellés est fort ancienne; il est difficile de préciser les rapports exacts de ces quatre familles et leurs symbiontes ne nous renseignent guère à ce sujet car les grands groupes de Flagellés étaient sûrement différenciés avant la séparation des Blattes et des Termites.

L'immense famille des Termitidae naquit probablement vers le milieu des temps secondaires, sans doute à partir de quelques Rhinotermitidae, et a subi une évolution très complexe; tout en gardant le régime xylophage, les Termitidae ont perdu très tôt leurs Flagellés symbiotiques, mais ont dû acquérir pour digérer la cellulose une flore bactérienne complexe et variée. Si une microflore très riche s'observe dans l'intestin postérieur de *tous* les Termitidae il serait hasardeux d'affirmer qu'il s'agit toujours du même type d'association; des recherches de physiologie comparée s'imposent.

Outre ce type d'association très générale, les divers phylums des Termitidae en ont acquis d'autres. Le cas le plus clair est celui des Termites champignonnistes; la sous-famille des Macrotermitinae s'est séparée assez tôt des autres Termitidae, mais il n'est pas sûr que l'association avec les champignons ait été acquise d'emblée. En effet, dans la faune actuelle, *Sphaerotermes sphaerothorax* construit des meules où ne végète *aucun champignon*[33]; nous tenons ce Termite pour une forme primitive de Macrotermitinae, et nous admettrions volontiers qu'il s'est différencié avant l'acquisition des champignons symbiotiques, mais après l'acquisition du comportement constructeur des meules. Au contraire, Emerson[34] pense plutôt que *Sphaerotermes* aurait perdu son associé par une évolution régressive.

Les Bactéries du segment intestinal mixte et les quelques cas analogues constituent aussi une association bien caractérisée, quoique nous ne soyons nullement certains de son caractère symbiotique. Tous les cas observés ont-ils une origine commune? Nous n'osons pas l'affirmer, car le segment mixte pourrait bien s'être différencié parallèlement dans les diverses lignées qui le possèdent aujourd'hui; l'hypothèse d'une convergence ne peut pas être écartée.

Les phénomènes de convergence sont certains dans le cas des Amibes. Si nous ne considérons que les Amibes xylophages ou humivores, on les rencontre en effet chez beaucoup de Termitinae, quelques Nasutitermitinae et enfin chez les Blattes du genre *Panesthia*! Nul doute qu'il ne s'agisse de commensaux ou de symbiontes d'acquisition relativement récente et indépendante.

Enfin, les *Termitophrya* paraissent s'être différenciés dans une lignée bien précise d'Apicotermitinae; les trois genres *Jugositermes, Rostrotermes, Trichotermes* sont en effet étroitement apparentés, comme en témoigne la structure du tube digestif et singulièrement la valvule entérique[16], mais bien distincts des autres Apicotermitinae. La présence de ces Ciliés étranges chez *Pericapritermes* paraît au contraire être secondaire, car ce genre appartient à une autre sous-famille (Termitidae) et les Protistes s'y localisent dans le rectum et non dans une dilatation spéciale du proctodéum. Il s'agit sans aucun doute d'un passage secondaire (désertion à partir de l'hôte original) dans un nouvel hôte.

L'extrême uniformité morphologique des Isoptères masque en fait une évolution complexe, des adaptations très poussées, non seulement dans le comportement social mais aussi dans la physiologie individuelle.

Les associations symbiotiques montrent bien l'importance de cette évolution physiologique et en jalonnent les principales étapes. Les grandes lignes de ces phénomènes commencent à nous apparaître; il faut maintenant les analyser et en préciser les caractères adaptatifs.

Summary

An attempt is made to retrace the evolution of symbiotic associations in Blattopteroidea, with particular reference to Isoptera. It seems probable that double symbiosis occured in the common ancestor of Blattids and Termites: intracellular microorganisms of bacteriocytes, xylophagous intestinal Flagellates. Where as actual Blattids, excepting *Cryptocercus*, have only retained the former, the primitive Termites, excepting *Mastotermes*, have only kept the latter. Evolved Termites (fam. Termitidae) have in turn lost the symbiotic Flagellates, but have acquired a rich and complex bacterial flora. – Some of these microorganisms, mixed with their aliments, certainly aid digestion (breakdown of cellulose), whilst others, localised at a particular level of the alimentary canal and not found in the alimentary mass, presumably play a different rôle. Furthermore, Protozoa are constantly associated with certains Termites (xylophagous Amoebae, humus-feeder Ciliates of the genus *Termitophrya*) and may play some rôle in the process of digestion. Finally, an association occurs between fundgus-growing Termites (subfam. Macrotermitinae) and mushrooms (*Termitomyces*).

[33] P.-P. Grassé et Ch. Noirot, Ann. Sci. nat. Zool. *10*, 149 (1948).

[34] A. E. Emerson, Amer. Mus. Novit., No. 1771, p. 31 (1956).

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Biophysikalische Studien mit synthetischem Lezithin

Wir haben kürzlich gezeigt[1], dass synthetisches Dipalmitoyl-α-lezithin[2], in Chloroform gelöst, speziell bei Gegenwart von zweiwertigen Kationen wie Kalzium, Barium usw., beim Schütteln mit einer neutralen wässerigen Lösung von Tropäolin dieses der wässerigen Phase entzieht und in die Chloroform-Phase überführt. Dabei wurde der Grad der Aktivierung des Lezithins durch verschiedene Kationen miteinander verglichen.

Es hat sich nun gezeigt, dass Lezithin, in CCl_4 gelöst, unter sonst gleichen Bedingungen weniger aktiv ist als in Chloroform, dass also nicht nur die Gegenwart bestimmter Ionen in der wässerigen Phase, sondern auch die Eigenschaft des lipoiden Lösungsmittels die Aktivität des Lezithins massgebend beeinflusst. Dass Lezithin in apolaren Lösungsmitteln wie Benzol in Form von Mizellen[3] oder in einer dielektrisch unwirksamen Form[4] vorliegt, ist bereits früher auf anderen Wegen erkannt worden. Dass diese Mizellen auch in unserem Versuch in CCl_4 weniger wirksam sind, deutet darauf hin, dass ihre polaren Gruppen sich in apolaren Lösungsmitteln zu heteropolaren Ringen vereinigen, so dass sowohl ihre Dipolmomente wie auch ihre Additionsfähigkeit für Fremdionen verschwindet. Im Hinblick auf die zweifellos überragende biologische Bedeutung des Lezithins und die Tatsache, dass dieses jeweils zusammen mit andern Lipoiden wie Fetten, Wachsen, Cholesterin usw. vorkommt, erschien es uns von Interesse zu untersuchen, durch welche neutralen lipophilen Stoffe Lezithin in apolarer Phase (CCl_4) in unserem Versuch aktiviert, das heisst diese Mizellen gelockert werden, so dass es bereits ohne die Gegenwart von Kalzium[1] usw. direkt Tropäolin-Natrium anlagert.

Wir haben, in Anlehnung an die in unserer ersten Mitteilung[1] beschriebene, sehr einfache Versuchsanordnung jeweils in 4 cm³ einer Lösung von 73 mg% Lezithin[5] in frisch destilliertem CCl_4 0,4 cm³, bzw. 0,4 g der zu prüfenden Substanz, gelöst und mit 4 cm³ einer 5 mg-proz. Tropäolin-Na-Salz-Lösung in $^1/_{500}$ *M* Phosphatpuffer vom pH 7,2 in einem dickwandigen Reagensglas 4 min kräftig geschüttelt, dann zentrifugiert und in der klaren überstehenden wässerigen Lösung das darin verbliebene Tropäolin spektrophotometrisch bestimmt. Wir erhielten für die homologen primären Alkohole die in Tabelle I ersichtlichen relativen Werte. Als Mass der Aktivierung des Lezithins dient die Abnahme an Tropäolin in der wässerigen Phase in % des ursprünglich vorhandenen.

Die Unwirksamkeit des Methylalkohols rührt zweifellos daher, dass er beim Schütteln in die wässerige Phase übergeht, da an diesem Test zugleich der Verteilungskoeffizient CCl_4/Wasser berücksichtigt wird.

Tabelle I

Alkohole	Abnahme an Tropäolin in der wässerigen Phase in %
Methanol	0 %
Äthanol	4,8%
n-Butanol	15,2%
n-Amylalkohol	20,0%
n-Heptylalkohol	14,0%
n-Oktylalkohol	3,6%
n-Nonylalkohol	0 %
n-Amylalkohol ohne Lezithin	0 %

Der Abfall der Wirkung bei den höheren Gliedern ist dem verhältnismässig geringeren Anteil an polaren Hydroxylgruppen zuzuschreiben, und es resultiert daher ein flaches Maximum der Wirkung in der Gegend des Amylalkohols.

Tabelle II

Ketone	
Aceton	2,0%
Methyl-Äthylketon	8,0%
Di-Äthylketon	30,0%
Di-Isobutylketon	0 %
Acetophenon	0 %
Cyclopentanon	23,0%
Cyclohexanon	17,2%
Cyclohexanon, bei 50°C geschüttelt	6,8%

Tabelle III

Säureamide u. a.	Abnahme an Tropäolin in der wässerigen Phase in %
Benzonitril	0 %
Chlorbenzol	0 %
Nitrobenzol	0 %
Benzoesäureäthylester	0 %
Zimtsäure-*n*-amylamid	0 %
p-Toluolsulfonsäurediäthylamid	0 %
Zimtsäuredimethylamid	10,6%
Cinnamalessigsäurepiperidid	15,6%
Zimtsäurediäthylamid	16,3%
Zimtsäurediäthylamid, bei 50–60° C geschüttelt	8,6%
Zimtsäurepiperidid	17,6%
p-Methoxyzimtsäurepiperidid	21,8%
Zimtsäuremorpholid	29,0%
Zimtsäuremorpholid, bei 50–60° C geschüttelt	7,2%

[1] R. Hirt und R. Berchtold, Exper. *14*, 436 (1958).
[2] R. Hirt und R. Berchtold, Pharm. Acta Helv. *33*, 349 (1958).
[3] P. H. Elworthy, J. chem. Soc. *1959*, 813. – M. Faure and J. Legault-Demare, Bull. Soc. Chim. biol. *32*, 509 (1950). – H. I. Price and W. C. M. Lewis, Biochem. J. *23*, 1030 (1929). – E. Baer *et al.*, J. cell. comp. Physiol. *17*, 355 (1941). – K. J. Palmer und F. O. Schmitt, J. cell. comp. Physiol. *17*, 385 (1941).
[4] R. Kuhn, Chem. Ber. *68*, 2386 (1935).
[5] Synthetisches α-β-Dipalmitoyl-lezithin.

Ähnlich verhalten sich Ketone, die in Tabelle II aufgeführt werden.

Ein weiteres Beispiel einer homologen Reihe aktiver Verbindungen ist in Tabelle III dargestellt. Es sind dies N-Dialkylamide der Zimtsäure. Die Verhältnisse liegen ähnlich wie bei den Alkoholen, nur dass die Wirkung allgemein etwas erhöht erscheint.

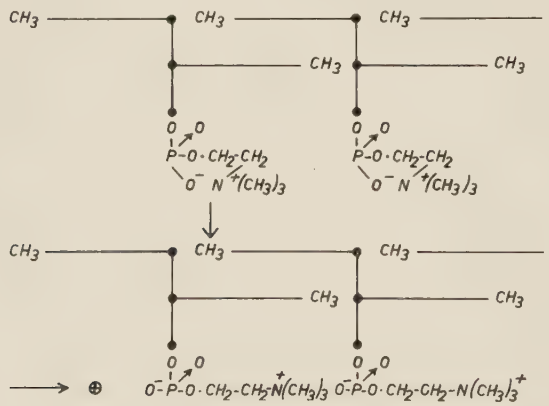
Dass es nun nicht die direkte Erhöhung der Dielektrizitätskonstante der lipoiden Phase ist, welche das Lezithin aktiviert, erhellt daraus, dass Nitrobenzol, Benzonitril, Chlorbenzol trotz hoher Dielektrizitätskonstante völlig unwirksam sind. Vielmehr ist anzunehmen, dass diejenigen Stoffe wirksam sind, welche die lipoide Phase hydratisieren, das heisst Wasser in sie einführen. Aktive Säureamide sind in kaltem Wasser viel leichter löslich als in warmem, was ebenfalls für eine Hydratisierung spricht. Besonders deutlich zeigen diesen Löslichkeitscharakter zum Beispiel die Diäthylamide der Buttersäure, Valeriansäure, Capronsäure und Dipropylamide der Krotonsäure. Die in diesem Versuch aktive Form des Lezithins ist wohl dessen Hydratform, wie sie von Baer[6] und auch von Howton[7] beschrieben worden ist und die wir durch Hydratwasser-Bestimmung nach Karl Fischer bestätigen konnten. Eigenartigerweise scheint das Gleichgewicht

$$\text{Lezithin-}(H_2O) \underset{n}{\rightleftarrows} \text{Lezithin}$$

in CCl_4 allein auch beim Schütteln mit Wasser ganz auf Seite der hydratwasserarmen Form zu liegen, und erst die Zugabe eines Lösungsvermittlers für Wasser ermöglicht die Bildung der stärker hydratisierten Form.

Diskussion der Ergebnisse. Nach Kuhn[4] ist Lezithin in benzolischer Lösung völlig intermolekular heteropolar assoziiert und dielektrisch unwirksam, während es in Alkohol monomolekular vorliegt. Man darf wohl annehmen, dass in einem ganz bestimmten Milieu diese beiden Formen sehr leicht ineinander übergehen können, was uns, zusammen mit unseren Ergebnissen und der Tatsache, dass Lezithin sehr leicht Lamellen und Schläuche (sog. Myelin-Figuren) bildet, veranlasst, die folgenden Hypothesen aufzustellen:

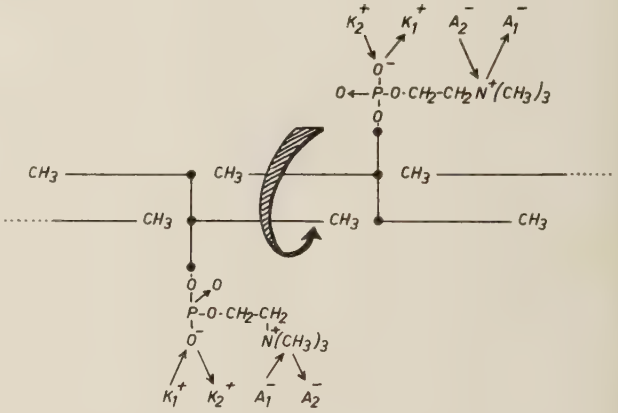
1. Darnach könnte man sich vorstellen, dass das Prinzip der Nervenleitung in folgendem Vorgang besteht:



Eine Lezithin-Lamelle oder ein -Röhrchen mit der im 1. Bild dargestellten oberen labilen Struktur wird bei der Annäherung einer positiven Ladung, zum Beispiel Proton oder anderes Kation, von links unter Umlagerung von der intramolekularen zur intermolekularen Zwitterionverknüpfung in die untere Form übergehen, wobei auf der rechten Seite die fortgeleitete positive Ladung in Form der Trimethylammoniumgruppe wieder erscheint. Von rechts nach links kann natürlich ebenso eine negative Ladung geleitet werden. Diese Leitung wäre eine dielektrische Verschiebung einzelner Impulse über die Kette und wäre verknüpft mit einer kleinen materiellen Verschiebung der zwitterionischen Ladungsträger $-O^-\text{-}N(CH_3)_3^+$ Dadurch wäre die gegenüber der metallischen Leitung sehr viel geringere Geschwindigkeit der Nervenleitung plausibel, ebenso die Tatsache, dass der Nerv nur einzelne Impulse bei einer bestimmten Reizschwelle überleiten kann.

Massgebend für die Funktion dieser Leitung ist die dynamische Situation. Fixierung des Lezithins in einer der beiden Extremformen durch zu starke oder zu schwache Hydratisierung, hervorgerufen durch Eindringen geeigneter Stoffe in das Medium, müsste diese Leitung hemmen.

2. Die Fähigkeit des Lezithins, aus wässeriger Phase ionisierte Stoffe zu addieren und sie in lipophile Komplexe überzuführen, legt weiter den Gedanken nahe, Lezithin könnte als «rotating carrier[8]» in folgender Weise eine wesentliche Rolle beim aktiven Transport ionogener Substanzen durch die Zellwand spielen:



Lamellenförmig in der schematisch angegebenen Form aneinandergelagerte Lezithinmoleküle der Zellwand drehen sich um die Längsachse ihrer Fettsäureketten; beladen sich auf der einen Seite mit Anionen A_1 und Kationen K_1 und tauschen nach der Drehung auf der andern Seite die Ionen gegen A_2 und K_2 aus und drehen sich damit wieder zurück.

Die isolierende Fettschicht würde durch diese Drehung niemals unterbrochen, um so mehr als ohne weiteres mehrere solcher Lamellen übereinandergelagert werden können, wobei stafettenartig die Ionen von einer Schicht auf die andere übertragen werden. Es ist klar, dass lipoidlösliche Stoffe ohne diesen Mechanismus diese Fettschicht durchdringen können. β-Lezithin, das biologisch offenbar nicht vorkommt, wäre unfähig, diese Drehung auszuführen. Durch Temperaturerhöhung wird die Hydratisierung der aktivierenden Stoffe sowohl in wässeriger wie in lipoider Phase vermindert. Dies bewirkt eine Verminderung der Aktivität des Lezithins bei erhöhter Temperatur in unserem Versuch. Spielt nun die Aktivität des Lezithins eine bestimmte Rolle im Zellstoffwechsel, so wäre über die mehr oder weniger starke Hydratisierung der Lipoide und damit parallel eine Veränderung der Lezithinaktivi-

[6] E. Baer, J. Amer. chem. Soc. *75*, 621, 5535 (1953).
[7] D. R. Howton, Science *119*, 420 (1954).
[8] J. F. Danielli, Soc. exper. Biol. *8*, 509 (1954).

tät zudem ein automatischer Mechanismus der biologischen Temperatursteuerung gegeben.

R. Hirt und R. Berchtold

Forschungsinstitut Dr. A. Wander AG., Bern, 29. Mai 1959.

Summary

Dissolved in an apolar solvent (CCl_4), lecithin, also when shaken with water, is present in an inactive form of low water content. Certain liposoluble substances hydratize the lipid phase and consequently the lecithin dissolved therein, converting it into the active form.

The importance of lecithin for cellwallpermeability and for conduction of nerve impulses is discussed.

Über die Trennung von Phenol-Derivaten an Ionenaustauscherharzen

Über erfolgreiche Trennungen von Phenol und seinen Derivaten auf papierchromatographischem Wege ist verschiedentlich berichtet worden[1]. Die Ausbeute der getrennten Verbindungen bleibt jedoch im μg-Bereich; die quantitative Ermittlung lässt sich daher relativ schlecht durchführen.

Eluierungsbild eines Gemisches von Phenolhomologen

In eigenen Untersuchungen ist die Möglichkeit geprüft worden, mit Hilfe von kernsulfonierten Polystyrolharzen, die eine hohe Kapazität besitzen, eine Trennung und Reinigung einzelner Phenolderivate zu erreichen. Wir verwendeten 55 X 1 cm-Säulen, die mit der Na-Form des Kationenaustauschers Dowex 50 X 4 (minus 400 mesh) gefüllt waren. Die Präparierung der Harze ist an anderer Stelle beschrieben worden[2,3].

Das Kationenaustauscherharz wird mit einem Na-Zitrat-Puffer vom pH 3,42 (2,4) in die chromatographische Röhre eingeschlämmt und mit etwa 120 ml 0,2 *n* NaOH gewaschen. Nach Äquilibrierung mit der genannten Pufferlösung ist die Säule gebrauchsfertig. Die Analysenprobe, die 10–15 mg jeder Komponente umfassen kann, soll einen pH-Wert von 3–3,4 besitzen. Die Eluierung erfolgt mit der angegebenen Pufferlösung bei einer Durchlaufgeschwindigkeit von 15 ml/h. Die Durchsatzrate der Pufferlösung kann bis auf 30 ml/h erhöht werden, ohne eine Verschlechterung des Trenneffektes zu bewirken, wenn entsprechend feinkörnige Harzpartikel verwendet werden. Die Einzelkomponenten haben wir mit Hilfe von diazotiertem Nitranilin sowie mittels der Infrarotspektroskopie qualitativ im Eluat nachweisen können.

Über weitere Untersuchungen zur Trennung von komplexen Gemischen von Phenolderivaten an Ionenaustauschersäulen wird an anderer Stelle berichtet werden.

G. Krampitz und W. Albersmeyer

Institut für Anatomie und Physiologie der Haustiere der Universität Bonn, 5. Mai 1959.

[1] F. Cramer, *Papierchromatographie*, 4. Auflage (1958), p. 149.

[2] S. Moore und W. H. Stein, J. biol. Chem. *152*, 663 (1951); *211*, 893 (1954).

[3] R. Müller und G. Krampitz, Z. Tierernährg. Futtermittelkunde *11*, 44 (1956).

Summary

A new method for the chromatographic separation of phenolic derivatives on sulphonated polysterene resins is reported.

Effet cytologique des traitements mécanique et ultrasonique sur quelques bacilles aérobies

Mudd *et al.*[1] ont soumis divers bacilles gram-positifs à l'action des vibrations soniques; après 10 min ils ont observés des fragments gram-négatifs parmi les bactéries gram-positives et ont utilisé ce procédé pour l'isolement des parois cellulaires. Cependant la plupart des études similaires ont été faites avec un vibrateur électromagnétique spécial (Mickle[2]) en combinaison avec des «ballotini» (perles de verre) de 0,2 mm de diamètre; cette technique a surtout été employée pour l'isolement des parois cellulaires (Dawson[3], Salton et Horne[4]). D'une façon plus détaillée, Tomcsik et Baumann-Grace[5] ont étudié l'effet cytologique de cet appareil sur 32 souches de *B. megaterium*, 12 de *B. anthracis* et 31 de *B. cereus*. Le but du présent travail est d'élucider et de comparer les effets de ces 2 techniques sur les formes végétatives et les spores de quelques bacilles aérobies. Nous avons utilisé le vibrateur Mickle et un oscillateur ultrasonique M. S. E. Mullard.

1. *Effet des vibrations ultrasonique sur les formes végétatives de B. anthracis, B. cereus et B. megaterium* (*Bacillus M*). Les cultures sur le milieu Gladstone-Fildes gélosé ont été maintenues 16 h dans l'étuve a 30° C (*B. cereus* et Bacillus M) ou dans l'étuve a 37° C (*B. anthracis*). Le premier effet de l'agitation ultrasonique sur les formes végétatives est le découpage des chaînes: après un temps très court de 1 ou 2 min, toutes les formes sont monocellulaires. Le second effet se manifeste sur la paroi latérale: les parois deviennent vides, sont brisées en fragments de plus en plus petits et après 30 min leur dissolution est presque complète. De cette observation, nous pouvons tirer la conclusion que les parois transversaux sont scindées avant la lésion des parois latérales. L'effet sur la capsule de Bacillus M a été également étudié: après 90 s d'agitation ultrasonique le serum antipolysaccharidique ne révèle plus de septums transversaux mais des condensations polaires sont encore visibles; le serum antipolypeptidique montre une destruction partielle de la capsule. Après 5 à 10 min 80% des formes monocellulaires sont vides mais l'encre de Chine révèle encore la présence de la couche intérieure de la capsule. Après 20 min, la dissolution des cellules est très avancée. La capsule ne protège donc pas, d'une façon appréciable, la paroi cellulaire.

Bacillus M ne forme des spores que sur l'extrait de pommes de terre gélosé mais même après 14 jours d'incubation sur ce milieu, on n'observe pas de libération des spores (Tomcsik et Baumann-Grace[6]). Si l'on sou-

Travail effectué avec l'aide du Fonds de recherches Hans Buess.

[1] S. Mudd, K. Polevitzky, T. F. Anderson et L. A. Chambers, J. Bact. *42*, 251 (1941).

[2] H. Mickle, J. R. micr. Soc. *68*, 10 (1948).

[3] J. M. Dawson, *The Nature of the Bacterial Surface* (Blackwell, Oxford 1949), p. 119.

[4] M. R. J. Salton et R. W. Horne, Biochim. biophys. Acta *7*, 19 (1951).

[5] J. Tomcsik et J. B. Baumann-Grace, Schweiz. Z. Path. Bakt. *19*, 566 (1956).

[6] J. Tomcsik et J. B. Baumann-Grace, Schweiz. Z. Path. Bakt. *21*, 914 (1958).

met de telles cultures à la désintégration ultrasonique, on voit, comme avec les formes non sporulées, tout d'abord un découpage des chaînes, ensuite une destruction des parois latérales. Bien que ces parois soient probablement influencées enzymatiquement, elles ne sont pas plus rapidement dissoutes que celles des formes végétatives: après 20 min, une partie des parois est solubilisée mais une autre partie persiste sous la forme de débris; les spores sont libres, parfaitements intactes.

2. *Effet des procédés sur les spores de quelques souches de B. cereus.* Après 1 a 2 h d'agitation dans l'oscillateur sonique, les spores de 2 souches de *B. cereus* sont restées réfringentes, intactes mais l'examen dans l'encre de Chine et l'addition d'un serum antispores de *B. cereus* a révélé que l'exosporium était presque complètement dissout. Par ce procédé il sera donc possible de séparer l'exosporium de la paroi des spores et nous espérons que l'isolement de l'exosporium facilitera les études chimiques et immunologiques de cette couche superficielle des spores.

Nous avons comparé l'effet des vibrations ultrasonique et de l'agitation mécanique sur une mélange de spores de diverses souches de *B. cereus*. Après 45 min d'agitation dans l'oscillateur sonique, toutes les spores ont conservé leur rétringence mais l'exosporium est presque complètement dissout. Après la même durée d'agitation dans le vibrateur Mickle toutes les spores sont noires ou vides mais ont gardé leur exosporium. La moitié de cette dernière suspension est de nouveau agitée durant 45 min dans le vibrateur Mickle: on peut encore voir beaucoup de spores vides avec leur exosporium et la suspension reste trouble; l'autre moitié est agitée durant 45 min dans l'oscillateur sonique: il n'est plus possible d'identifier une seule paroi de spores même après l'addition du serum antispores de *B. cereus* et la clarification de la suspension est très nette. Une agitation préalable de 45 min dans le vibrateur ultrasonique ne semble pas augmenter la sensibilité des spores de *B. cereus* à la désintégration mécanique dans le vibrateur Mickle.

J. Tomcsik et Mélina Bouille

Institut d'Hygiène de l'Université de Bâle (Suisse), le 28 juillet 1959.

Zusammenfassung

Ultraschall-Behandlung der in Ketten gewachsenen aeroben Bazillen spaltet vorerst die Querwände des Zellverbandes, wodurch in 60 s intakte, monozellulare Formen entstehen. Die «vegetative» Zellwand wird erst nach einigen Minuten geschädigt; sie wird dann je nach Spezies in 20–60 min mehr oder weniger aufgelöst, wobei die Kapsel weder bei der Mickle- noch bei der Überschallbehandlung nennenswerte Schutzwirkung entfaltet. Die von den vegetativen Zellresten befreiten, bakteriellen Endosporen bleiben bei der Ultraschall-Behandlung, im Gegensatz zur Mickle-Behandlung, selbst nach 2 h intakt; ihr Exosporium wird aber nach etwa 1 h aufgelöst. Diese Beobachtung ermöglicht die Isolierung des Exosporiums und das serologische und chemische Studium dieser bisher nicht klar definierten Sporensubstanz. Durch alternierende Behandlung: Ultraschall-Mickle-Ultraschall kann auch die Sporenwand, frei vom Exosporium, in Lösung gebracht werden.

Der Einfluss von Chlorpromazin auf die oxydative Phosphorylierung von Tumormitochondrien

In früheren Untersuchungen[1,2] konnten wir zeigen, dass Trijodthyronin in *hoher* Konzentration auf den Fundamentalprozess der mit der Atmung gekoppelten oxydativen Phosphorylierung störend einwirkt. Die glykolysierende Karzinomzelle erleidet eine stärkere energetische Schädigung als ökonomisch arbeitende, normale proliferierende Zellen wie das Knochenmark usw. Experimentelle und klinische Beobachtungen zeigten eindrucksvolle Regressionen verschiedener Tumorarten[3]. Da aber TJTh Nebenerscheinungen zeigte, prüften wir Chlorpromazin (als «Megaphen» der Firma Bayer) auf seine Wirksamkeit innerhalb unserer Kombinationsmöglichkeiten.

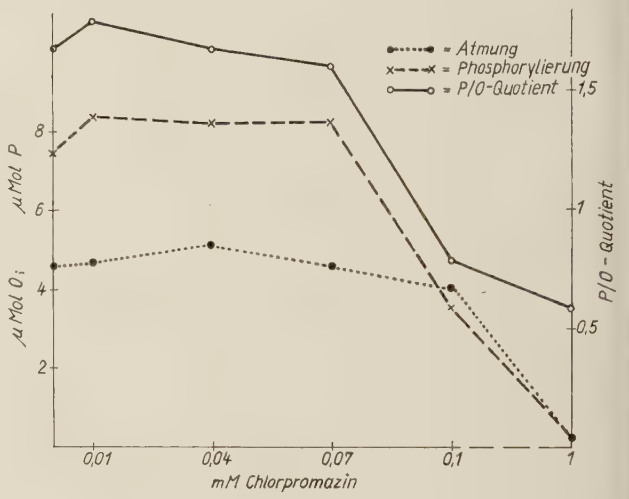


Abb. 1. Einfluss von Chlorpromazin auf die Atmung, Phosphorylierung und den P/O-Quotienten von Lebermitochondrien. Medium: 50 µ*M* Phosphat; 60 µ*M* $MgCl_2$; 30 µ*M* NaF; 150 µ*M* Glukose; 0,4 mg Hexokinase; 150 µ*M* KCl; 30 µ*M*-Ketoglutarsäure; 5 µ*M* ATP; 0,3 ml Mitochondrien (1–1,5 mg N) in 0,25 *M* Zucker- und 0,002 *M* EDTA-Lösung; pH 7,0. Endvolumen: 3,0 ml; Temperatur 30°C; Zeit: 15 min.

Vom Chlorpromazin ist bekannt, dass es die Atmung von isolierten Lebermitochondrien der Ratte bei gleichzeitiger Verminderung der Phosphorylierung herabsetzt[4–7]. Im Gegensatz zu Löw[7] fanden wir, dass Chlorpromazin in *geringen* Konzentrationen von 10^{-8} bis 10^{-5} *M* eine Steigerung der Atmung und Phosphorylierung bei isolierten Lebermitochondrien der Ratte erkennen lässt. Es fand sich eine Zunahme des P/O-Quotienten (Abb. 1).

Wir untersuchten den Einfluss von Chlorpromazin auf die oxydative Phosphorylierung von Tumormitochondrien und fanden eine deutliche Beeinflussung der P/O-Quotienten schon bei geringen Chlorpromazindosen.

[1] W. Lührs, E. Heise und G. Bacigalupo, Nature, *183*, 1534 (1959).

[2] G. Bacigalupo, W. Lührs und E. Heise, Acta biol. med. german. *2*, 455 (1959).

[3] W. Lührs, 7. Deutsch. Krebskongress, April 1959 in Berlin (im Druck).

[4] H. B. Collier und G. M. Allenby, Can. J. med. Sci. *30*, 443 (1952).

[5] L. G. Abood, Proc. Soc. exp. Biol. Med., N.Y. *88*, 688 (1955).

[6] M. Berger, H. J. Strecker und H. Waelsch, Nature *177*, 1234 (1956).

[7] H. Löw, Biochim. biophys. Acta *32*, 11 (1959).

Abbildung 2 zeigt die Atmung, Phosphorylierung und den P/O-Quotienten von isolierten Mitochondrien des Ehrlich-Mäuse-Ascites-Tumors unter der Einwirkung geringer Konzentrationen von Chlorpromazin.

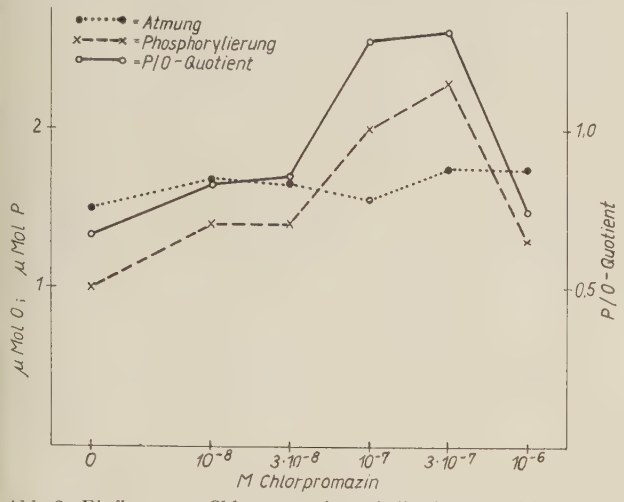


Abb. 2. Einfluss von Chlorpromazin auf die Atmung, Phosphorylierung und den P/O-Quotienten von Mäuse-Ascites-Tumor-Mitochondrien. Medium: 50 μM Phosphat; 60 μM $MgCl_2$; 30 μM NaF; 75 μM Glukose; 0,2 mg Hexokinase; 5 μM ATP; 0,02 μM Zytochrom c; 3 μM DPN; 30 μM-α-Ketoglutarsäure. 0,3 ml Mitochondrien (1,2 mg N) in 0,25 M Zucker- und 0,002 M EDTA-Lösung; pH 7,0. Endvolumen: 3,0 ml; Temperatur: 30°C; Zeit: 20 min.

Interessant war das Ergebnis einer gleichen Versuchsanordnung bei isolierten Mitochondrien des Walker-Karzinoms der Ratte (Abb. 3). Hier fand sich eine unterschiedliche Wirkung höherer Konzentrationen des Chlorpromazins gegenüber der Stoffwechselwirkung auf normale Lebermitochondrien (siehe Abb. 1 zum Vergleich).

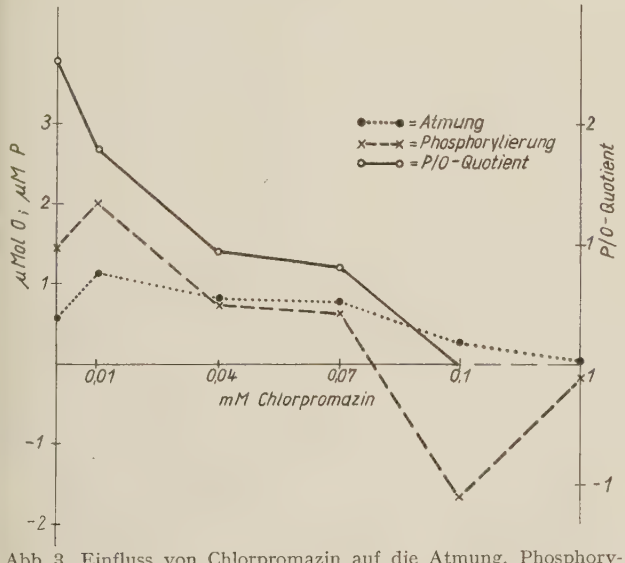


Abb. 3. Einfluss von Chlorpromazin auf die Atmung, Phosphorylierung und den P/O-Quotienten von Walker-Karzinom-Mitochondrien. Bedingungen wie in Abbildung 1.

Die in den Abbildungen verwendeten Abkürzungen sind: ATP = Adenosin Triphosphorsäure; ADP = Adenosin Diphosphorsäure; DPN = Diphosphor-Pyridin-Nukleotid; EDTA = Äthylen-Diamin-Tetraessigsäure.

Man kann aus diesen Ergebnissen folgern, dass durch höhere Konzentrationen von Chlorpromazin der P/O-Quotient der Tumormitochondrien wesentlich stärker herabgesetzt wird als der von normalen Lebermitochondrien. *In vivo* bei transplantierbaren Tiertumoren erhielten wir Ergebnisse, die unsere *in-vitro*-Befunde bestätigen. Hierüber wird an anderer Stelle berichtet. Gleiche Ergebnisse wurden unter gleichen Versuchsbedingungen mit einer anderen entkoppelnden[8] Substanz, dem Atebrin, erhalten.

Auf Grund unserer ersten klinischen Erfahrungen bei der kombinierten Behandlung des humanen Krebses mit Cytostatica oder Bestrahlung möchten wir folgern, dass Tumor-Mitochondrien gegenüber denen von gesunden, auch proliferierenden Mausergeweben eine grössere Beeinflussbarkeit durch Stoffe zeigen, die die oxydative Phosphorylierung je nach Konzentration steigern oder mindern können. Für das Chlorpromazin möchten wir feststellen, dass hohe Konzentrationen dieses Stoffes durch die nachweisbare Entkopplung der oxydativen Phosphorylierung die schon energetisch schwache Tumorzelle in ein Energiedefizit versetzen, so dass zusätzlich verabreichte Cytostatica oder energiereiche Strahlen selektiver wirken können. Beim krebskranken Menschen wirken sich die bekannten anderen pharmakologischen Effekte des Chlorpromazins günstig aus.

W. Lührs, G. Bacigalupo,
B. Kadenbach und E. Heise

Abteilung für experimentelle und klinische Chemo-Therapie der Geschwulstklinik des Institutes für Medizin und Biologie der Deutschen Akademie der Wissenschaften in Berlin-Buch, 12. Mai 1959.

Summary

High concentrations of chlorpromazine cause a considerable decrease of the oxidative phosphorylation of tumor mitochondria and rat liver mitochondria. Regarding preliminary experimental and clinical investigations, we assume that malignant tumors can be sensitised to cytotoxic substances and ionising radiations impairing their energy metabolism by uncoupling the oxidative phosphorylation.

[8] H. Löw, Biochim. biophys. Acta *32*, 1 (1959).

Immune Electrophoretic Analysis of Bovine Milk and Purified Bovine Milk Protein Fractions

The analysis of proteins by means of diffusion-in-gel techniques has been shown to be advantageous because of the sensitivity and specificity of these methods[1]. As a part of a study of bovine milk proteins from chemical and immunological points of view an investigation of the antigenic composition of bovine milk and some purified proteins from milk has been undertaken by means of the Ouchterlony plate technique and immune electrophoresis. Similar studies on human milk have recently been published by Hanson and Johansson[2–4].

Mature bovine milk was used throughout this study. Immune sera against the milk were obtained from hyper-

[1] P. Grabar, Adv. Protein Chem. *13*, 1 (1958).
[2] B. Johansson, Nature *181*, 996 (1958).
[3] L. Å. Hanson and B. Johansson, Int. Arch. Allergy (in press).
[4] L. Å. Hanson, Int. Arch. Allergy (in press).

immunized rabbits. Immune electrophoretic analyses were performed according to Grabar and Williams[5]. In some instances the micro-modification described by Scheidegger[6] was used. Controls of absorption experiments were performed with the micro-slide technique of Wadsworth[7].

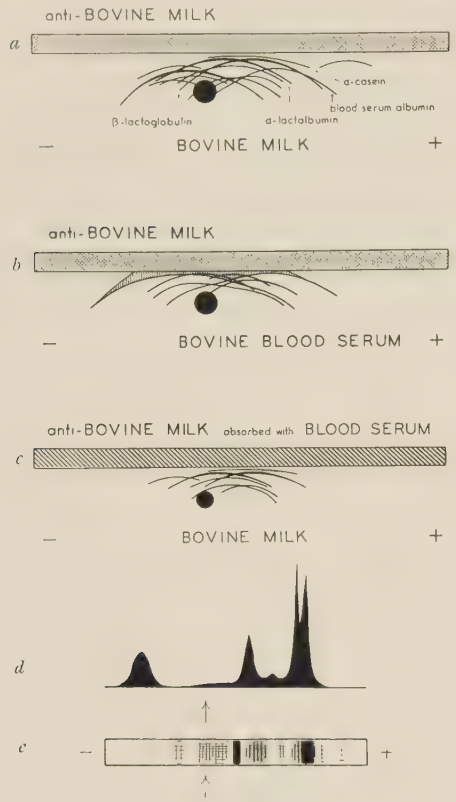


Fig. 1

a) Diagram of immune electrophoretic analysis of bovine milk by means of anti-bovine milk serum.
b) Diagram of immune electrophoretic analysis of bovine blood serum by means of anti-bovine milk serum.
c) Diagram of immune electrophoretic analysis of bovine milk by means of anti-bovine milk serum absorbed with bovine blood serum.
d) Moving boundary electrophoresis of bovine milk.
e) Agar gel electrophoresis of bovine milk.

The milk-anti-milk spectrum obtained in an immune electrophoretic experiment is shown in Figure 1*a*. With the immune serum used, twelve separate precipitates were formed. The relative mobilities of the antigenic factors varied from that of the immune globulins to a mobility higher than serum albumin as determined by the position of the immune precipitates when compared with moving boundary electrophoresis and agar gel electrophoresis of the milk (Fig. 1*d* and *e*). The immune electrophoretic analysis of bovine blood serum by means of the anti-milk serum showed six separate precipitation lines (Fig. 1*b*). These were situated throughout the regions of γ-globulin to serum albumin as shown by comparison with agar gel electrophoresis of bovine blood serum. The presence of antigenic factors in the milk corresponding serologically to blood serum albumin and blood serum γ-globulin was indicated by absorption experiments. Anti-milk immune serum was absorbed with a preparation of blood serum albumin and used to analyze electrophoretically separated milk and blood serum. In both experiments the precipitates observed in the albumin regions in the total spectra (Fig. 1*a* and *b*) had vanished. In a similar manner anti-milk immune serum was absorbed with a preparation of blood serum γ-globulin and tested with electrophoretically separated milk and blood serum. Thus the line found in the γ-globulin region of the total anti-milk-blood serum spectrum (Fig. 1*b*) and the most dense precipitate situated in the immune globulin region of the milk-anti-milk spectrum (Fig. 1*a*) were both absent when this absorbed antiserum was employed.

The anti-milk serum, absorbed with bovine blood serum, showed seven separate precipitation lines in the immune

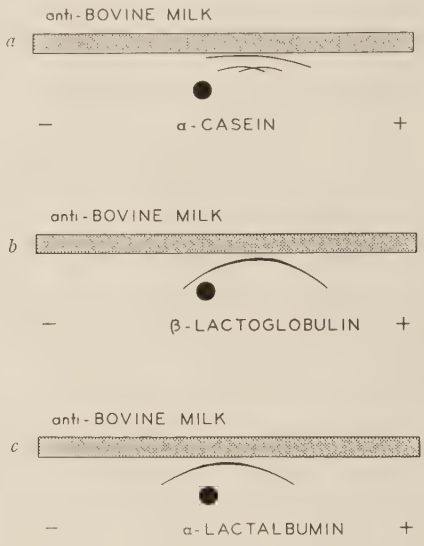


Fig. 2

a) Diagram of immune electrophoretic analysis of α-casein by means of anti-bovine milk serum.
b) Diagram of immune electrophoretic analysis of β-lactoglobulin by means of anti-bovine milk serum.
c) Diagram of immune electrophoretic analysis of α-lactalbumin by means of anti-bovine milk serum.

electrophoretic analysis of bovine milk (Fig. 1*c*). This spectrum was assumed to contain the dominating milk proteins, i.a. α-casein, β-lactoglobulin and α-lactalbumin. In order to identify these proteins in the precipitation spectra, preparations of α-casein[8], crystalline β-lactoglobulin[9], and crystalline α-lactalbumin[9] were made and tested by immune electrophoresis. The patterns formed by these preparations are shown in Figure 2*a–c*. The α-casein preparation gave a long line extending from the starting basin to the albumin region. The line had no distinct maximum. When a higher concentration of α-casein was used two more lines were formed as seen in Figure 2*a*. The β-lactoglobulin and α-lactalbumin preparations each gave one line situated in the regions corresponding to the localization of these proteins in agar gel electrophoresis. Experiments with the double diffusion technique, also showed three lines with α-casein and one line each with β-lactoglobulin and α-lactalbumin. Comparative analysis of the α-casein with blood serum albu-

[5] P. Grabar and C. A. Williams, Biochim. biophys. Acta *17*, 67 (1955).
[6] J. J. Scheidegger, Int. Arch. Allergy *7*, 103 (1955).
[7] C. Wadsworth, Int. Arch. Allergy *10*, 355 (1957).
[8] N. J. Hipp, M. L. Grovers, J. H. Custer, and T. L. McMeekin, J. Dairy Sci. *35*, 272 (1952).
[9] R. Aschaffenburg and J. Drewry, Biochem. J. *65*, 273 (1957).

min and β-lactoglobulin by means of anti-milk serum indicated that these three substances were serologically unrelated. However, analysis of α-lactalbumin and β-lactoglobulin with their homologous sera showed more than one precipitate with each protein. By means of absorption experiments the position of the β-lactoglobulin and α-lactalbumin precipitation lines in the total milk-anti-milk spectrum could be established as designated in Figure 1*a*. In the same way the extended line formed by α-casein could be identified in the total spectrum.

These and further investigations of the immunological characteristics of bovine milk proteins in native and modified forms will be published in detail elsewhere.

L. Å. Hanson and B. Johansson

Departments of Bacteriology and Medical Biochemistry, University of Gothenburg (Sweden), May 6, 1959.

Zusammenfassung

Immunoelektrophoretisch liessen sich aus Kuhmilch mit Hilfe einiger Kaninchen-Anti-Milchseren mindestens 12 Immunpräzipitate nachweisen. Von diesen waren 6 Präzipitate serologisch mit Blutserumproteinen verwandt (u. a. Albumin und γ-Globulin). Ferner wurden Präparate von α-Kasein, β-Laktoglobulin und α-Laktalbumin analysiert.

Catalase Activity in the Regenerating Tail Tip of Xenopus Larvae and the Effect of 3-Amino-1,2,4-triazole[1]

In previous papers[2,3] we were able to show that tail tissue of Xenopus larvae contains a catalase with much the same kinetic properties as catalase from mammalian organs[2]. It is distributed along the tail axis in a steep gradient, the specific activity being highest in the tip and lowest in the base of the tail[3]. Catalase is well known for its marked depression in the livers and kidneys of tumor-bearing rats, whereas it shows normal levels of activity in regenerating rat liver[4]. On the other hand we have found recently that the activity of the intracellular proteases (cathepsins) is strongly increased above the normal level in the regenerating tail tip of Xenopus larvae; it is further increased in regenerates partly inhibited by a morphostatic aminoketone and a quinoxaline derivative[5]. In view of this, it was of interest to follow the behaviour of catalase activity during the process of regeneration in Xenopus larvae. This would extend our knowledge of the enzyme pattern in the regenerating tail tissue and serve as a basis for further tests of the biochemical effects produced by morphostatic substances on our model tissue.

[1] This work was supported by a grant of the *Eidgenössische Kommission zur Förderung der wissenschaftlichen Forschung aus Arbeitsbeschaffungsmitteln des Bundes*. I would like to express my gratitude to Prof. F. E. Lehmann for his constant interest and his valuable advice. I am also indebted to Mrs. J. Weber and to Miss M. Pieren for technical assistance.

[2] H. P. von Hahn, Helv. chim. Acta *42*, 49 (1959).

[3] H. P. von Hahn, Exper. *14*, 67 (1958).

[4] J. P. Greenstein, *Biochemistry of Cancer* (Academic Press Inc., Publ., New York 1954), p. 519.

[5] H. P. von Hahn and F. E. Lehmann, Helv. physiol. Acta *16*, 107 (1958).

In the first test we determined the effect of 3-amino-1,2,4-triazole (AT), known as a potent catalase inhibitor *in vivo* in the rat[6], on our system.

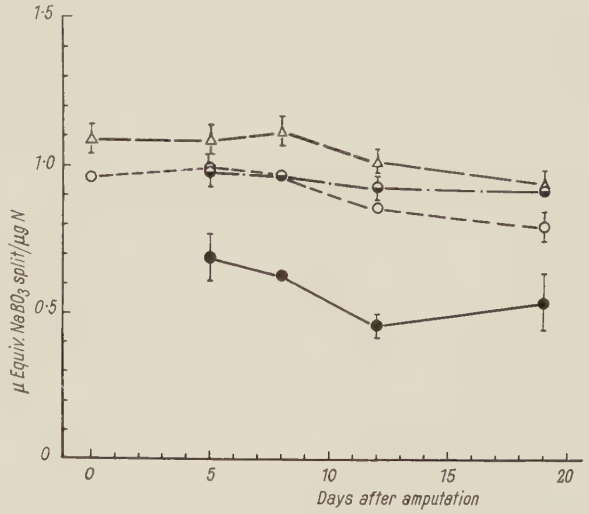


Fig. 1. – Specific catalase activity during the regeneration of the tail tip of Xenopus larvae. ● Regenerates, ⊖ stumpslices, ▽ 0th–7th mm and ○ 7th–9th mm of the tails of the unamputated controls. The vertical lines indicate the standard error of the determinations.

Methods. We used the micro-adaptation of the perborate test of Feinstein[7] which we recently described[2]. Xenopus larvae, 25 days old and 26–30 mm in overall length, were kept in distilled water at 18°C in a thermostat. In the first group of animals, 7 mm of the tail tip were amputated at the beginning of the experiment (day 0). A second group of non-amputated animals served as a control and was kept under identical conditions. The larvae were not fed throughout the period of the experiment. On the 5th, 8th, 12th, and 19th days after amputation, tissue pieces were collected from at least 10 larvae each, as follows: (a) from the amputated animals, the regenerates and the adjoining 2 mm stump slices; (b) from the non-amputated animals 7 mm slices from the tail tip as controls to the regenerates and 7th–9th mm slices as controls to the stump slices. Catalase activity and total nitrogen (by the ultramicro-Kjeldahl technique of Boell and Shen[8]) were determined in homogenates of these 4 tissue pieces.

In the experiment with AT, the amputated larvae were kept in a 1:4000 solution of AT. One group of non-amputated larvae in AT and another in water served as controls. The regenerates and the 2 mm stump sclices were collected from the amputated animals, and the 7th–9th mm slices were collected from the tails of the treated and the untreated controls. The results for both experiments are expressed in microequivalents perborate split per µg total nitrogen (for an incubation time of 5 min).

Results. Specific catalase activity during normal regeneration is shown in Figure 1. In the tails of the non-amputated controls catalase activity remained almost unchanged throughout the experimental period, the tip

[6] W. G. Heim, D. Appleman, and H. T. Pyfrom, Science *122*, 693 (1955); Amer. J. Physiol. *186*, 19 (1956). – R. N. Feinstein, S. Berliner, and F. O. Green, Arch. Biochem. Biophys. *76*, 32 (1958). – E. Margoliash and A. Novogrodsky, Biochem. J. *68*, 468 (1958).

[7] R. N. Feinstein, J. biol. Chem. *180*, 1197 (1949).

[8] E. J. Boell and S. C. Shen, Exp. Cell Res. *7*, 147 (1954).

of the tail to the 7th mm showing consistently about 10% more activity than the adjoining 2 mm slice (7th–9th mm). This confirms the gradient-like distribution shown previously[3]. The slight loss of activity in these two tissue pieces from the 8th to the 19th day is not statistically significant. The regenerates, on the other hand, show a marked decrease in catalase activity to about 50% of the level found in the corresponding region of the non-amputated tails (0–7 mm). Only towards the end of the regeneration period does the activity seem to increase again, to a very slight extent. That this decrease is specific for the regenerating tissue is shown by the unchanged level of activity in the adjoining 2 mm stump slices. This behaviour is in contrast to the results found in regenerating rat liver, where catalase activity is not decreased[4]. It is also in contrast to our own results with the cathepsins[5], which in the regenerates return to the normal level within 10 days after amputation, i.e. when the regenerates are fully differentiated and further growth proceeds without gain in nitrogen content[9]. In the case of catalase, the abnormally low level is maintained, and in this respect the regenerates keep their specific characteristics even after differentiation is completed.

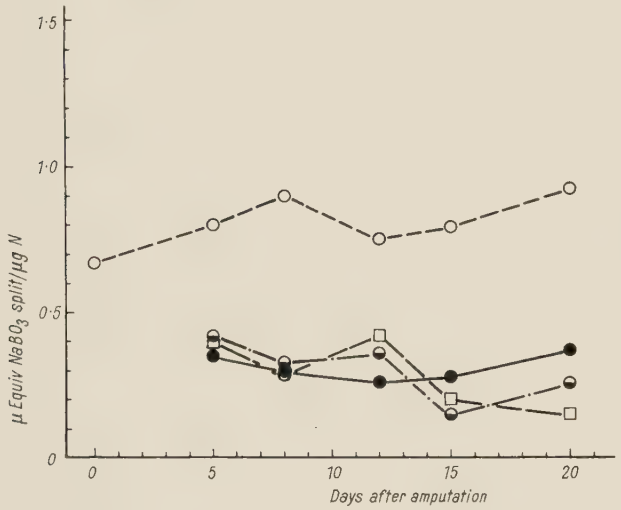


Fig. 2.—Specific catalase activity during regeneration in a 1:4000 solution of AT. ● Regenerates, ◒ stump slices, □ 7th–9th mm of the tails of the unamputated larvae treated with AT, ○ 7th–9th mm of the tails of the untreated controls.

A concentration of 1:4000 of AT inhibited catalase in the tail tissue of amputated and non-amputated larvae (Fig. 2). Both in the regenerates and in the stump slices of the amputated larvae as well as in the 7th–9th mm slices of the treated, non-amputated controls the activity was depressed to about the same level, namely 20–40% of the untreated controls. In the regenerates, catalase activity was decreased to a much smaller extent than in the stump and control slices. Here the level falls only to about 60 to 70% of the untreated regenerates (see Fig. 1). It seems as if at the AT concentration used, a definite minimum level of catalase activity is maintained in the treated tissues.

The regeneration of tail tissue was partly inhibited by AT during the first days after amputation. On the 5th day, AT-treated regenerates measured only 0,55 mm (average of 50) while untreated regenerates measured 1,00 mm (average of 10). But by the 12th day treated regenerates averaged 3,99 mm to 3,78 mm for the untreated, and at the end of the experiment (20th day) the values were 5,20 and 4,45 mm respectively. Thus it seems that those cells in the young blastema which survived the first effects of the treatment retained their full vitality, since the tissue as a whole did not lose any of its regenerative power.

One is led to the conclusion that the blocking of catalase in the regenerate does not in itself affect any vital metabolic pathway, and that the inhibition observed may possibly be due to a contaminating substance with a specific effect on the early phases of regeneration. The presence of such biologically active 'aminotriazole contaminant' has been discussed by Feinstein *et al.*[10]. It should be possible to separate the catalase-blocking and the regeneration-inhibiting components of AT-preparations and thus identify the active agents. Such work is at present in progress and will be reported on later.

H. P. von Hahn

Zoologisches Institut der Universität Bern, June 17, 1959.

Zusammenfassung

In der regenerierenden Schwanzspitze der Xenopuslarve bleibt die Katalase-Aktivität (bezogen auf Totalstickstoff) bis zum Ende der Regenerationsperiode auf etwa 50% des normalen Niveaus, während sie im anschliessenden Schwanzstumpf unverändert ist. 3-Amino-1,2,4-triazol erniedrigt die Katalase-Aktivität in den Regeneraten, den Stümpfen und dem Kontrollgewebe nicht-amputierter Larven auf etwa 20–40% des normalen Niveaus. Es bewirkt eine kurzfristige Regenerationshemmung in den ersten Tagen nach Amputation, die im weiteren Verlauf wieder vollständig ausgeglichen wird.

[9] E. M. Deuchar, R. Weber, and F. E. Lehmann, Helv. physiol. Acta *15*, 212 (1957).

[10] R. N. Feinstein, S. Berliner, and F. O. Green, Arch. Biochem. Biophys. *76*, 32 (1958).

Die Komplement-hemmende Wirkung einiger Antiproteasen

Zum Wirkungsmechanismus des Komplements bei immunologischer Hämolyse gehört eine primäre Aktivierung von C'_1 durch den Antigen-Antikörper-Komplex (eventuell auch eine unspezifische Aktivierung durch die proteolytischen Enzyme Plasmin und Trypsin) zu «aktivem C'_1», welches die Eigenschaften einer Protease mit Esterase-Aktivität aufweisen soll[1–3]. Die Hemmung dieser ersten Stufe und damit des ganzen Komplements könnte für Allergie und Immunohämatologie von grosser Bedeutung sein[3,4]. Cushman *et al.*[5,6] beobachteten die hemmende Wirkung von Substanzen wie *p*-Toluolsulfonyl-l-argininmethylester, einigen Polypeptiden, Aminosäuren und Diisopropylfluorophosphat; diese waren jedoch entweder zu wenig aktiv oder wegen ihrer Toxizität therapeutisch nicht verwendbar.

[1] E. L. Becker, J. Immunol. *77*, 462 (1956).
[2] E. L. Becker, J. Immunol. *77*, 469 (1956).
[3] E. L. Becker, J. All. *29*, 3 (1958).
[4] H. Fischer, W. Fritsche und H. Argenton, Klin. Wschr. *36*, 411 (1958).
[5] W. F. Cushman, E. L. Becker und G. Wirtz, J. Immunol. *79*, 80 (1957).
[6] L. Levine, Biochim. biophys. Acta *18*, 283 (1955).

Wir prüften die Komplement-hemmende Wirkung einiger Antiproteasen aus Pankreas, Sojabohnen, Phaseolus, Kartoffeln und Ovomucoid sowie den Einfluss der IV. Äthanolfraktion des Plasmas, indem die Verminderung oder Aufhebung der Lyse von sensibilisierten Hammelerythrozyten durch Zugabe dieser Stoffe beobachtet wurde.

Methodik. – Hammelerythrozyten. Hammelblut wurde steril in die Alsever-Lösung entnommen und durch Schütteln mit Glasperlen defibriniert. Die abzentrifugierten Erythrozyten wurden anschliessend zweimal mit physiologischer Kochsalzlösung und einmal mit Veronalpuffer von pH 7,4[7] gewaschen. Zur Standardisierung wurde eine 5%ige Blutkörperchensuspension hergestellt, die 10^6 Erythrocyten pro ml enthalten sollte. Die Sensibilisierung erfolgte durch den Ambozeptor (1 ml Suspension + 1 ml Puffer pH 7,4 + 4–8 Ambozeptoreinheiten wurden 10 min bei 37°C inkubiert), dann wurde die Suspension auf eine Konzentration von 0,4% verdünnt.

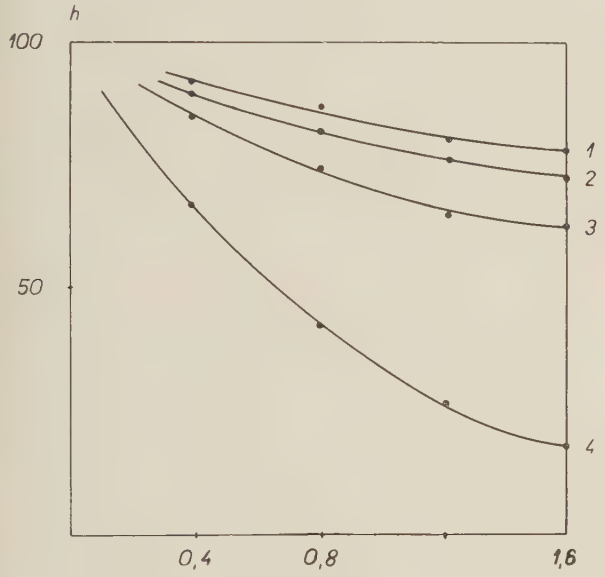


Abb. 1. Hemmende Wirkung von Antiproteasen auf das hämolytische System. *1:* Inhibitor aus Kartoffeln, *2:* aus Pankreas, *3:* aus Phaseolus, *4:* aus Sojabohnen; *h:* Hämolyse in Prozenten (Hämolyse ohne Inhibitor = 100%), *i:* Konzentration des benützten Inhibitors in %.

Komplement. Als Komplementquelle diente humanes RP-Serum, das heisst Serum, aus welchem das Properdin mit Hilfe von Zymosan entfernt worden war[8].

Antiproteasen. Es wurden Inhibitoren aus Pankreas und Phaseolus[9], der teilweise gereinigte Inhibitor aus Sojabohnen, der chromatographisch gereinigte Inhibitor aus Kartoffeln[10], die IV. Plasmafraktion nach Cohn sowie Ovomucoid verwendet. Die Substanzen wurden in Veronalpuffer von pH 7,4 gelöst. Zum hämolytischen System wurde von dieser Lösung so viel zugegeben, dass die zu prüfenden Substanzen in Konzentrationen von 0–1,6 g% vorhanden waren. Die Antitrypsin- und Antiplasminaktivitäten der untersuchten Antiproteasen sind in Abbildung 3 und 4 wiedergegeben.

[7] L. Pillemer, L. Blum, I. H. Lepow, O. A. Ross, E. W. Todd und A. C. Wardlaw, Science *120*, 279 (1954).

[8] H. C. Isliker und E. Linder, Vox Sang. *3*, 23 (1958).

[9] V. Mansfeld, A. Ziegelhöfer, Z. Horáková und J. Hladovec, Naturwiss. *46*, 172 (1959).

[10] V. Mansfeld und J. Hladovec, im Druck (1959).

Zur Feststellung der *Antiplasminaktivität* wurde die Hemmung der durch Plasmin[11] bedingten Lyse des Fibrinkoagulums gemessen.

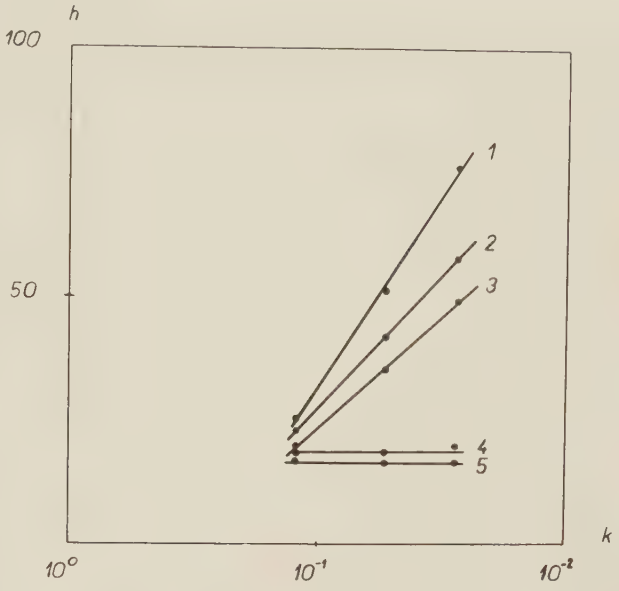


Abb. 2. Komplementhemmende Wirkung von Antiproteasen und Properdin in Gegenwart von Zymosan bei 37°C. Inaktivierung des Komplements durch Rattenproperdin (*1*); Inaktivierung des Komplements durch Rattenproperdin + Antiprotease aus Pankreas (*2*), Phaseolus (*3*), Sojabohnen (*4*), Kartoffeln (*5*). Konzentration der Inhibitoren: 0,8%; *h* = Hämolyse; *k* = Konzentration des Properdins.

Die *Antitrypsinaktivität* wurde aus dem Grad der Hemmung einer proteolytischen Spaltung von Kasein bestimmt[12].

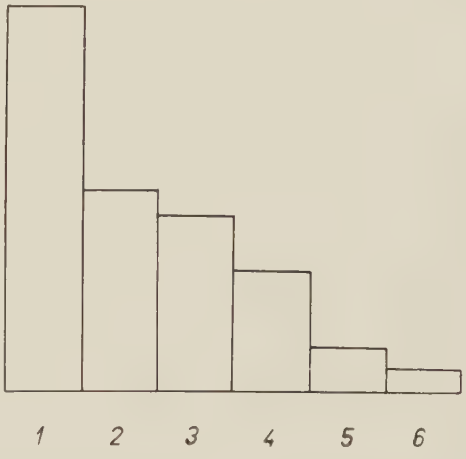


Abb. 3. Relative Hemmung von Trypsin durch Antiproteasen. Der Inhibitionseffekt der Pankreasantiprotease wurde als Basis genommen und die Wirkung der übrigen Inhibitoren im Verhältnis zum Pankreasinhibitor bestimmt. *1* = Inhibitor aus Pankreas, 2 = Ovomucoid, *3* = aus Sojabohnen, *4* = aus Phaseolus, *5* = aus Kartoffeln, *6* = IV. Fraktion von Humanplasma.

Properdin. Als Properdinquelle wurde Rattenserum verwendet und die Properdinaktivität nach Isliker[8] bestimmt.

[11] M. Rybák und J. Rejnek, Clin. chim. Acta *4*, im Druck (1959).

[12] J. Hladovec und V. Mansfeld, Coll. Czech. chem. Comm. *23*, 1796 (1958).

Versuchsanordnung. In die Reagensgläser wurden folgende Lösungen pipettiert:

0,1 ml RP-Serum
0–0,4 ml Inhibitor
0,4–0 ml Puffer pH 7,4.

Das Volumen des Systems betrug immer 0,5 ml, die Konzentration des Inhibitors variierte von 0 bis 1,6 g%. Die Lösung wurde während 15 min bei 37°C inkubiert; dann wurden 2 ml Erythrozytensuspension zugefügt und das Gemisch weitere 30 min bei gleicher Temperatur gehalten. Nach der Inkubation wurde die Suspension zentrifugiert und photometrisch (Unicam) die Extinktion gemessen. Eine Hämolyse ohne Inhibitorzugabe wurde als 100% angenommen (Abb. 1).

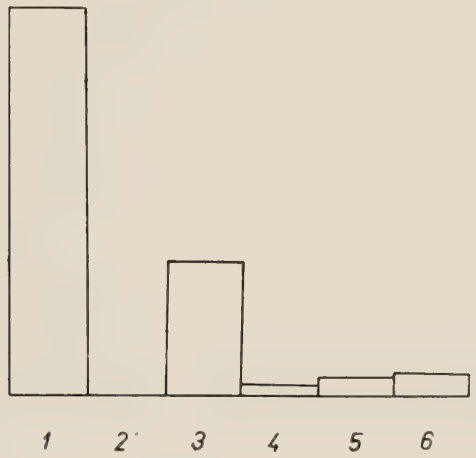


Abb. 4. Relative Hemmungswirkung von Antiproteasen auf Plasmin. Der Inhibitionseffekt der Antiprotease aus Pankreas wurde als Basis genommen und die Wirkung der übrigen Inhibitoren im Verhältnis zum Pankreasinhibitor bestimmt. *1* = Inhibitor aus Pankreas, *2* = Ovomucoid, *3* = aus Sojabohnen, *4* = aus Phaseolus, *5* = aus Kartoffeln, *6* = IV. Fraktion von Humanplasma.

Die Antiproteasewirkung auf das hämolytische System äusserte sich in einer Hemmung der prozentualen Lyse, wie Abbildung 1 und 2 zeigen. Die intensivste Wirkung besass der aus Sojabohnen hergestellte Inhibitor, während die aus Pankreas, Phaseolus und Kartoffeln gewonnenen Präparate eine relativ geringere Hemmung ergaben. Ovomucoid war ohne jeglichen Effekt. Die native IV. Plasmafraktion zeigte eine Properdin-hemmende Wirkung, die nach Erwärmung auf 60°C allmählich verschwand.

Die hemmende Wirkung von Properdin auf C'_3 in Gegenwart von Zymosan bei 37°C wurde durch die Inhibitoren mit Ausnahme der IV. Fraktion nicht gestört; die resultierende Hämolyseverminderung setzte sich aus der inaktivierenden Wirkung des Properdins und der Komplementhemmung durch die Antiproteasen zusammen. Bei Inhibitoren aus Sojabohnen und Kartoffeln überstieg die aktuelle Inhibition die Summe beider Inaktivierungswirkungen (Abb. 2).

Die hier beobachtete Komplement-Hemmung durch Antiproteasen liefert einen weiteren Beweis für den Proteasencharakter mindestens einer Komplementkomponente (C'_1). Wir sind ferner der Ansicht, dass dieser Inhibitionseffekt in der Therapie einiger allergischer Erkrankungen und vielleicht auch der toxischen hämolytischen Anämien ausgenützt werden könnte.

M. Rybak, M. Petakova, V. Mansfeld, and J. Hladovec

Institut für Hämatologie und Bluttransfusion und Forschungsinstitut für Pharmazie und Biochemie, Prag, 7. Mai 1959.

Summary

The complement-inhibiting action of some antiproteases (i.e. the inhibitors from pancreas, sojabeans, Phaseolus, potatoes, ovomucoid, and the effect of the IV. ethanol plasma fraction) has been observed. It was found that the antiproteases produced from sojabeans, pancreas, Phaseolus, and potatoes possess the inhibitory effect.

On the Distribution in Brain of Monoamines and of Enzymes Responsible for their Formation

In a previous investigation, the distribution of noradrenaline and dopamine in the brains of several mammalian species was mapped out[1,2]. High amounts of noradrenaline were detected in the hypothalamus and other parts of the brain stem, whereas dopamine was found to be localized almost exclusively in the nuclei of the corpus striatum of the cerebral hemispheres. The brain parts rich in catechol amines also proved to have high L-dihydroxyphenylalanine (DOPA) decarboxylase

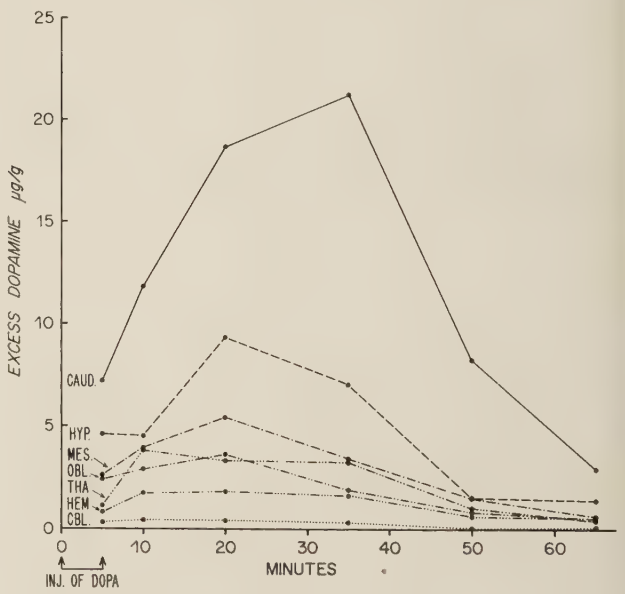


Fig. 1. – Excess concentrations of dopamine in different brain parts at varying intervals of time after injection of DOPA. Caud.: Caudate nucleus. Hyp.: Hypothalamus. Mes.: Mesencephalon. Obl.: Medulla oblongata + pons. Tha.: Diencephalon (not hypothalamus). Hem.: Hemispheres (not corpus striatum). Cbl.: Cerebellum

activities *in vitro*. These and other data seemed to indicate that the two amines were involved in the functions of different regions of the brain. The investigation has now been continued and some additional data are given in this paper.

It was thought of interest to study the distribution of noradrenaline and dopamine in some further detail. The

[1] Å. Bertler and E. Rosengren, Exper. *15*, 10 (1959).

[2] Å. Bertler and E. Rosengren, Acta physiol. Scand. (in press).

human brain seemed well suited for such studies, as the various structures of this brain can be more easily dissected out than in other species. The brain tissues were obtained from the post mortem room 6–16 h after death of the patients. They were examined for their noradrenaline and dopamine contents as described in earlier papers[2–4].

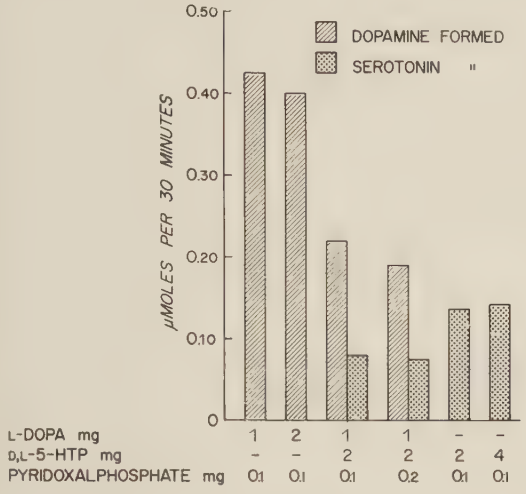


Fig. 2. — Decarboxylation of DOPA and 5-HTP and a mixture of the two amino acids by a hog brain extract. Each vessel contained 2·5 ml hog brain extract (pH 7·5) corresponding to 0·7 g tissue. Temperature 37° C. Final volume 3·1 ml

Considerable amounts of dopamine were found in the caudate nucleus and the putamen, which contained 2·0 to 6·5 µg/g. In the globus pallidus no dopamine could be detected. The highest concentration (0·8–1·9 µg/g) of noradrenaline in the human brain was observed in the rostral and intermediate portions of the hypothalamus. Lower values were recorded in the region of the mammillary bodies. The noradrenaline and dopamine values in these and other areas of human brain were about 30–50% of those observed in other species. The lower values in the human brain may be explained by the long interval between death and extraction of the tissues. It is interesting that dopamine is confined to cytologically similar nuclei which constitute the phylogenetically youngest part of the corpus striatum. Noradrenaline and 5-hydroxytryptamine (5-HT) are localized mainly in older areas of the brain.

The uneven distribution of catechol amines in brain has prompted a study of the capacity of different brain parts to form and inactivate such amines. To this end DOPA, which is the precursor of dopamine, was injected intravenously into normal adult rabbits in a dose of 100 mg DL-form/kg. The duration of injection was 5 min. The animals were killed at varying intervals of time after the end of the injection by a blow on the neck and the blood was collected. The brains were divided into the various parts and the corresponding parts from two animals analyzed together. The results are given in Figure 1. It will be seen that after administration of DOPA, dopamine accumulated in the brain, which is in accordance with earlier observations[5]. The increase varied, however, widely from one part of the brain to another. The rate of accumulation roughly parallelled the concentration of preformed catechol amines, i.e. it was highest in the caudate nucleus and the hypothalamus, somewhat lower in the rest of the brain stem, and still lower in the cerebral hemispheres and the cerebellum. The excess concentrations of dopamine reached their maxima 15–30 min after the end of the DOPA injection and then decreased at rates suggesting a first order reaction. When plotted on a semilogarithmic paper, the slopes of the declining parts of the curves were practically the same for the different parts of the brain. The ability to destroy dopamine thus appears to be the same throughout the brain, in agreement with the fact that the distribution of monoamine oxidase in brain is fairly uniform[6].

In a similar manner we have studied the accumulation of 5-HT after the injection of 5-hydroxytryptophan (5-HTP). The highest values of excess 5-HT were found in the hypothalamus and the caudate nucleus, the lowest in the cerebral hemispheres and the cerebellum[7].

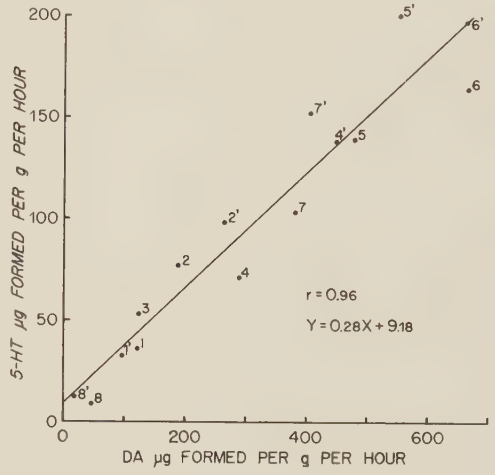


Fig. 3. — 5-HTP vs. DOPA decarboxylase activities in different parts of rabbit brain.

1. Hemispheres (not corpus striatum); 2. Caudate nucleus; 3. Lentiform nucleus; 4. Diencephalon (not hypothalamus); 5. Hypothalamus; 6. Mesencephalon; 7. Medulla oblongata + Pons; 8. Cerebellum. 1 and 1′ etc. represent data from two different experiments

The interpretation of the above data is partly dependent on the specificity of the DOPA and 5-HTP decarboxylases, a problem which has not yet been settled[9–11]. We have observed that DOPA decarboxylase in hog kidney cortex extracts was competitively inhibited by 5-HTP

[3] Å. Bertler, A. Carlsson, and E. Rosengren, Acta physiol. Scand. *44*, 273 (1958).

[4] A. Carlsson and B. Waldeck, Acta physiol. Scand. *44*, 293 (1958).

[5] A. Carlsson, M. Lindqvist, T. Magnusson, and B. Waldeck, Science *127*, 471 (1958).

[6] S. Udenfriend, P. A. Shore, D. F. Bogdanski, H. Weissbach, and B. B. Brodie, Recent Progr. Hormone Res. *13*, 1 (1957).

[7] In these experiments the examination of the different brain parts for their 5-HT contents was performed with a procedure which has been in use in this laboratory. The extraction and purification was principally carried out as described for catechol amines[3], except that another type of ion exchange resin was used, Amberlite XE-64 instead of Dowex 50. The final determination was performed fluorimetrically as described by Udenfriend[8].

[8] S. Udenfriend, H. Weissbach, and D. F. Bogdanski, Science *122*, 972 (1955).

[9] C. T. Clark, H. Weissbach, and S. Udenfriend, J. biol. Chem. *210*, 139 (1954).

[10] E. Westermann, H. Balzer, and J. Knell, Arch. exp. Path. Pharmak. *234*, 194 (1958).

[11] A. Yuwiler, E. Geller, and S. Eiduson, Arch. Biochem. Biophys. *80*, 162 (1959).

and *vice versa*. In other words, the data suggested that DOPA and 5-HTP were competing for one and the same enzyme. Experiments performed with hog brain extracts gave similar results. In Figure 2, the data from a typical experiment are given, in which an extract from the caudate and lentiform nuclei, the diencephalon and the mesencephalon was used. It will be seen that the addition of one of the amino acids caused an inhibition of the decarboxylation of the other. The inhibition was 50% when the concentrations of the L-forms of the two amino acids were equal. The inhibition does not seem to be due to lack of co-enzyme, as doubling the amount of pyridoxal-5-phosphate had no effect on the results.

A similar competition possibly also occurs *in vivo:* Repeated injections to rabbits of DOPA (600 mg/kg in total) during 2 h lowered the brain 5-HT level to 50–60% of the normal. Whether this decrease was due to inhibited biosynthesis or to release followed by oxidation, remains to be discovered, however.

In order further to elucidate this problem, DOPA and 5-HTP decarboxylase activities in various regions have been determined using the same technique as described earlier[2]. The results are given in Figure 3. A certain degree of correlation between the initial accumulation of dopamine after DOPA injection and DOPA decarboxylase activity is apparent from a comparison of Figures 1 and 3. A high order of correlation was found between the distribution of the two amino acid decarboxylases. This finding lends additional support to the assumption that the decarboxylation of the two amino acids is carried out by one and the same enzyme.

After the injection of DOPA, the animals showed increased motor activity and signs of sympathetic stimulation. The effect on motor activity appeared to be closely correlated to the accumulation of dopamine in brain: both phenomena appeared to reach their maxima in about 25 min. Motor activity then appeared to decline along with the drop in the dopamine level. About 50 min. after the injection of DOPA, there was still definite evidence of central excitation. It is interesting to note that at this interval the dopamine levels were fairly low in all parts of the brain except the corpus striatum, suggesting that this part of the brain forms an important site of action of dopamine on motor functions. Administration of 5-HTP caused an appearance quite different from that produced by DOPA. There was no motor hyperactivity, rather a decrease in voluntary movements combined with general muscle tremors.

This work has been supported by grants from the Swedish Medical Research Council and by the Air Force Office of Scientific Research of the Air Research and Development Command, United States Air Force.

Å. Bertler and E. Rosengren

Department of Pharmacology, University of Lund (Sweden), July 24, 1959.

Zusammenfassung

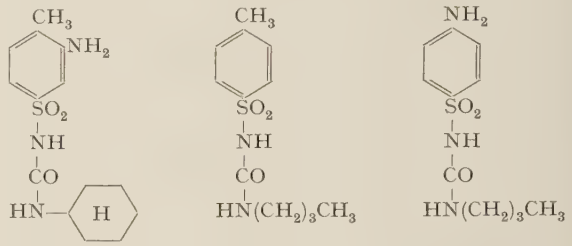
Im menschlichen Gehirn kommen grosse Mengen von 3-Hydroxytyramin im Nucleus Caudatus und dem Putamen vor.

Injektion von 3,4-Dihydroxyphenylalanin in Kaninchen bringt eine schnelle Akkumulation von 3-Hydroxytyramin in den Gehirnteilen, die reich an Katecholaminen sind, zustande. Die Fähigkeit das akkumulierte 3-Hydroxytyramin abzubauen scheint im ganzen Gehirn dieselbe zu sein.

Hemmungsversuche und Aktivitätsuntersuchungen der Fermente in verschiedenen Gehirnteilen *in vitro* sprechen dafür, dass 5-Hydroxytryptophan und 3,4-Dihydroxyphenylalanin von einem und demselben Ferment decarboxyliert werden.

On the Hypoglycemizing Effect of Metahexamide

Metahexamide (N(3-amino-4-methyl-benzenesulfonyl)-N'-cyclohexylurea) is a new oral hypoglycemizing agent[1] (see formula) having a better therapeutic index than other similar drugs.



Metahexamide D 860 BZ 55

Following the line of our previous investigations we have been interested to study the hypoglycemic action, the absorption and disappearance from blood and the effect on glucose uptake by rat diaphragm *in vitro*.

Methods. Metahexamide was always administered by stomach tube, as the reference drug *p*-tolylsulfonilbutylurea (D 860). Blood sugar levels were determined in duplicate in 0·1 ml samples by the Nelson procedure[2] in 12 h fasted Sprague-Dawley rats or rabbits of mixed breed.

Metahexamide has been evaluated in blood and kidney with a modified method of Bratton and Marshall[3]. To the filtrate obtained after precipitation with 15% trichloroacetic acid and filtration through no. 1 Whatman filter paper, were added 0·1% sodium nitrite (1 ml), 0·5% ammonium sulfamate (1 ml), concentrated hydrochloric acid (1 ml) and 0·1% N (1-naphthyl)ethylenediamine dihydrochloride (1 ml). The resulting colour was read in a photoelectric colorimeter using a filter having a maximum transmission at 520 mμ. Glucose uptake tests were performed on rat hemidiaphragms in Krebs-Ringer bicarbonate buffer as described elsewhere[4–6].

Results

a) *Hypoglycemic activity in vivo.* Metahexamide and D 860 (as reference drug) have been found to be active oral hypoglycemizing drugs both in rats and rabbits. In rats Metahexamide induces maximum blood glucose falls of 20, 35, and 50% respectively after 10, 40, and 100 mg/kg[7], while D 860 is without activity at 10 mg/kg and at 50 and 100 mg/kg, produces falls of blood sugar level to 35 and 50% respectively.

The maximum effect is achieved for both drugs about 2 h after administration, but Metahexamide hypoglycemia

[1] D. Müting, W. Presser, and K. Shivarom, Arzn.Forsch. *9*, 188 (1959).
[2] N. Nelson, J. biol. Chem. *153*, 375 (1944).
[3] A. Bratton and E. Marshall, J. biol. Chem. *128*, 537 (1939).
[4] C. R. Park *et al.*, Amer. J. Physiol. *182*, 12 (1955).
[5] N. Canal *et al.*, Clin. Terap. *11*, 472 (1956).
[6] S. Garattini *et al.*, Arzn.Forsch. *8*, 477 (1958).
[7] The LD_{50} of Metahexamide by oral route in rat is about 2 g/kg.

Table

No experiment	Control glucose uptake mg/g/h	Metahexamide		D 860		% Variation	Average
		γ/ml	Glucose uptake mg/g/h	γ/ml	Glucose uptake mg/g/h		
1	7·2	5	8·5	–	–	+22	
2	5·4	5	6·7	–	–	+24	22
3	7·0	5	8·4	–	–	+20	
4	5·6	10	6·9	–	–	+23·5	
5	6·0	10	7·7	–	–	+28·4	
6	4·5	10	5·4	–	–	+20·0	26
7	6·5	10	8·9	–	–	+36·9	
8	7·8	10	9·8	–	–	+25·7	
9	4·9	10	6·2	–	–	+26·5	
10	5·6	–	–	10	6·03	+ 7·7	
11	6·9	–	–	10	7·20	+ 4·3	1·7
12	4·3	–	–	10	4·00	− 7·0	
13	5·2	–	–	20	6·30	+21	
14	6	–	–	20	7·60	+26·7	
15	7·2	–	–	20	8·95	+24·5	24·8
16	6·6	–	–	20	8·20	+26·6	

is longer lasting than that of D 860, the first being present up to 8 h after administration. Similar results have been obtained in rabbits.

b) *absorption and excretion.* After a single dose of 40 mg/kg intraperitoneally in rat, the maximum blood concentration of Metahexamide was detected after 4 h (average 7·9 mg/100 ml), but also after 12 h a concentration of 6·4 mg/100 ml was present. The kidney Metahexamide concentration closely parallels that of blood, being 5·2 mg/100 g after 4 h and 4 mg/100 g, after 12 h.

The rapid absorption and the slow desappearance from blood of Metahexamide may account for the rapid onset of hypoglycemia and the long lasting effect.

c) *glucose uptake.* In the Table are reported the effects of Meta hexamide and D 860 on glucose uptake by rat hemidiaphragm *in vitro*.

As already shown for sulfanilylbutylurea (BZ 55) and D 860, Metahexamide also increases glucose uptake by isolated rat diaphragm. In this test Metahexamide is considerably more active than D 860.

From our findings we may conclude that the remarkable hypoglycemizing action of Metahexamide is justified by two properties: the slow metabolism and the strongly enhanced peripheral glucose utilization. These two effects could explain the higher hypoglycemic activity of Metahexamide in comparison with related compounds.

We want to thank Prof. C. Runti, Institute of Chemistry, University of Trieste, for the synthesis of Metahexamide

S. Garattini, A. Jori, V. Palma, and L. Tessari

Institute of Pharmacology, University of Milan (Italy), April 17, 1959.

Riassunto

La Metahexamide, N(3amino-4-metilbenzenesulfonil)N'-cicloesilurea, possiede nel ratto e nel coniglio un effetto ipoglicemizzante più narcato rispetto al *p*-tolilsulfanilbutilurea (D 860). Il nuovo farmaco si dimostra più attivo del D 860 nell'accelerare la scomparsa di glucosio da parte del diaframma isolato di ratto. La Metahexamide si distribuisce rapidamente in tutti gli organi e scompare dal sangue molto lentamente. Il maggiore effetto della Metahexamide, rispetto a composti similari, viene interpretato sulla base di un lento metabolismo e in rapporto all'intenso effetto favorente la metabolizzazione del glucosio a livello periferico.

Les glycoprotéines sériques dans le granulome expérimental provoqué par la carrageenine

Les travaux de Catchpole *et al.*[1] ont déjà établi une liaison entre la prolifération du tissu conjonctif dans les états inflammatoires et l'augmentation des mucoïdes sériques et urinaires. On sait d'autre part que l'haptoglobine constitue la fraction des globulines α_2 dont l'augmentation chez l'homme est parallèle à celle des mucoïdes perchloro- ou sulfosalicylosolubles dans de nombreux états pathologiques[2] (exception faite pour la plupart des tumeurs malignes[3] et certaines affections hépatiques[4]. Dans une revue générale l'un de nous et Boussier ont exposé les arguments en faveur de la production par le tissu cojonctif proliferé des mucoïdes α_1 et α_2 qui augmentent dans les états inflammatoires[5]. Cette augmentation constitue le reflet humoral de l'importance de la réaction inflammatoire de la substance fondamentale.

Nous avons entrepris une série d'études afin d'obtenir des renseignements d'ordre quantitatifs sur la teneur en mucopolysaccharides «neutres» du tissu conjonctif et sur les rapports qui existent entre ces mucopolysaccharides neutres et les glycoprotéines sériques. Dans ce travail nous étudierons le comportement des glycoprotéines sériques et en particulier celui de la fraction sulfosalicylosoluble et de l'haptoglobine au cours du développement du granulome expérimental.

[1] I. Gersch et H. R. Catchpole, Amer. J. Anat. *85*, 457 (1949). – M. B. Engel et H. R. Catchpole, Proc. Soc. exp. Biol. Med. N. Y. *84*, 336 (1953). – H. R. Catchpole, C. L. Pirani et A. Bestetti, Fed. Proc. *17*, 24 (1958).

[2] M. F. Jayle, J. Serpicelli et L. Robert, Clin. chim. Acta *1*, 452 (1956).

[3] L. Robert, J. Serpicelli et M. F. Jayle, Rev. Franç. Etud. clin. biol. *1*, 976 (1956).

[4] M. Nyman, Scand. J. clin. Lab. Invest. *11*, Suppl. 39 (1959).

[5] M. F. Jayle et G. Boussier, Exposés Ann. Biochim. méd. *17*, 157 (1955).

Tableau I

Comportement des glycoprotéines sériques du cobaye et du rat au cours du développement du granulome expérimental. Déterminations faites avant l'injection de la carrageenine et au bout du 6e jour après l'injection. Les chiffres représentent la moyenne obtenue sur 11 cobayes (de 400 à 600 g) et 8 rats (de 160 à 200 g) et l'erreur standard de la moyenne $\sigma/\sqrt{n}$. $\Delta\%$ représente la variation en % calculée par rapport à la valeur initiale

	Glycoprotéines totales mg acide sialique par 100 serum		$\Delta\%$	Glycoprotéines sulfosalicylosolubles mg acide sialique par 100 serum		$\Delta\%$	Glycoprotéines sulfosalicylosolubles mg p. 100 glycoprotéines totales		$\Delta\%$	Indice d'haptoglobine		$\Delta\%$
	avant	après		avant	après		avant	après		avant	après	
Cobaye .	98,6±4,6	105 ± 3,5	6,5	31,2 ± 4,2	42 ± 1,6	32,7	31,9 ± 4,5	40,2 ± 1,8	26	0,48±0,17	0,99±0,13	106
Rat . .	130 ±10,3	164 ± 4,8	26	23,1 ± 6,7	51,2 ± 6,0	122	19,7 ± 3,3	31,2 ± 3,1	58,5	0,59±0,18	2,43±0,31	312

L'haptoglobine (Hp) se prête plus facilement que d'autres glycoprotéines à une caractérisation qualitative et quantitative grâce à son affinité spécifique pour l'hémoglobine[5].

Le granulome expérimental a été provoqué par injection souscutanée de carrageenine dans la région abdominale des cobayes et des rats[6]. Nous avons utilisé la carrageenine de Seaplant Co.[7] après l'avoir précipitée 3 fois de suite par du propanol à partir de sa solution aqueuse[8]. On injecte 50 mg dans 5 ml de sérum physiologique, l'injection étant précédée d'une prise de sang dans le sinus rétroorbitaire, avec des capillaires héparinées[9]. Le 6e jour après l'injection de la carrageenine on refait une prise de sang, puis on tue les animaux, on dissèque le tissu de granulation formé et on procède aux analyses. Nous avons dosé l'acide sialique total du sérum par la méthode de Svennerholm[10] légèrement modifiée[11] ainsi que l'acide sialique du filtrat sulfosalicylique (0,5 ml de sérum, 1,5 ml de KOH 0,1 *N* et 2 ml d'acide sulfosalicylique à 20%; centrifugé au bout de 10 min). L'indice d'haptoglobine du sérum fut déterminé selon la méthode de l'un de nous[12] sur 0,02 à 0,1 ml de sérum. Le Tableau I montre les résultats. La comparaison statistique des données expérimentales est faite selon les recommandations de Lamotte[13]. Les résultats sont différents chez les cobayes et chez les rats.

On voit sur ce Tableau que les glycoprotéines sériques totales (mesurées par l'acide sialique) augmentent d'une façon significative après l'injection de carrageenine chez les rats. Cette augmentation s'explique très bien par celle de l'haptoglobine et celle des glycoprotéines sulfosalicylosolubles. Chez les cobayes par contre le taux des glycoprotéines totales reste inchangé aux erreurs d'expériences près; l'augmentation de la fraction sulfosalicylosoluble est moins forte que chez les rats (34% chez les cobayes, contre 122% chez les rats), mais significative à un coefficient de sécurité de 95%. L'haptoglobine augmente nettement chez les cobayes traités (106% d'augmentation en moyenne). Chez les rats l'augmentation de l'acide sialique total (34 mg/100 ml de sérum) est à peu de chose près égale à la somme de l'augmentation de l'acide sialique sulfosolicylosoluble et de l'acide sialique de l'haptoglobine (37,4 mg/100 ml de sérum). Il résulte des recherches de l'un de nous avec Boussier[5] qu'une unité de l'index d'haptoglobine correspond en chiffres rond à une concentration sérique de 1 mg Hp/ml et que la teneur en acide sialique de l'Hp est de 5%. Chez les cobayes l'acide sialique de la fraction sulfosalicylosoluble et de l'haptoglobine augmente en moyenne de 13,4 mg% pour une élévation de l'acide sialique total de 6,4 mg%. Il se peut donc que l'augmentation des glycoprotéines sulfosalicylosolubles et celle de l'haptoglobine soit accompagnée d'une baisse d'autres glycoprotéines. Winzler[14] et Weimer *et al.*[15] arrivent à des conclusions analogues au cours de l'étude des glycoprotéines perchlorosolubles chez les rats ayant reçu des implants de carcinome Walker cc256.

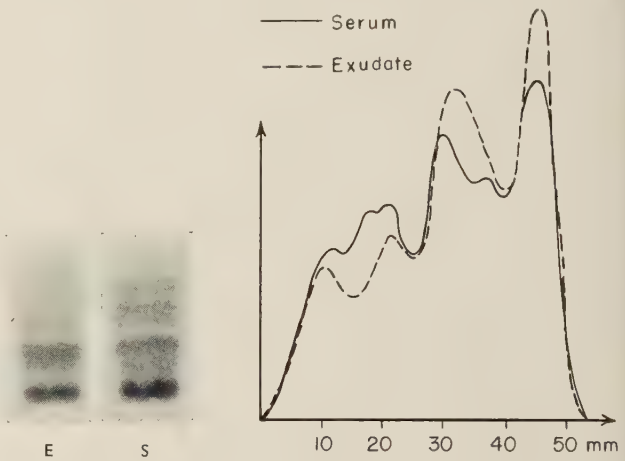


Diagramme d'électrophorèse sur papier du sérum et de l'exsudat de cobaye 6 jours après l'injection de carrageenine. L'exsudat est plus pauvre en β- et γ-globulines, un peu plus riche en albumine que le sérum. La forte bande en α de l'exsudat correspond au complexe hémoglobine-haptoglobine.

Le granulome des cobayes est le plus souvent riche en exsudat. Les déterminations effectuées sur 5 exsudats donnent une teneur nettement plus faible en acide sialique et en haptoglobine par rapport au sérum. Le Tableau II montre que cette différence disparaît si l'on ramène la teneur en acide sialique à 1 mg des protéines totales (déterminée par la réaction au biuret). C'est que l'exsudat est moins riche en protéines que le sérum: sa teneur en pro-

[6] W. van B. Robertson et B. Schwartz, J. biol. Chem. *201*, 689 (1953).

[7] New Bedford, Mass. USA. Nous tenons à remercier M. L. Stoloff, directeur de recherches du Seaplant Co., pour l'envoie gracieux d'une échantillon de carrageenine.

[8] L. Stoloff, Communication privée de Seaplant Co., New Bedford, Mass. U.S.A.

[9] B. N. Halpern et A. Pacaud, C. r. Soc. biol. *145*, 1465 (1951).

[10] L. Svennerholm, Biochem. biophys. Acta *24*, 604 (1957).

[11] L. Robert et J. Serpicelli, en préparation.

[12] M. F. Jayle, Bull. Soc. Chim. biol. *33*, 876 (1951).

[13] M. Lamotte, *Initiation aux méthodes statistiques en biologie* (Masson, Paris 1957), p. 81.

[14] R. J. Winzler, in *Methods of Biochemistry*, tome *2* (Ed. Glick, 1955), p. 279.

[15] H. E. Weimer, F. A. Quinn, J. Redlich-Moschin et H. Nishihara, J. nat. Cancer Inst. *19*, 409 (1957).

téines varie entre 18 et 55 mg/ml, celle du sérum entre 46 et 62 mg/ml.

Tableau II

Comparaison de la teneur en acide sialique et en haptoglobine du sérum et de l'exsudat chez le cobaye traité à la carrageenine. Les chiffres représentent la moyenne et l'erreur standard de la moyenne

	Indice d'hapto-globine	mg d'hapto-globine p. 100 mg protéines totales	mg acide sialique p. 100 ml	mg acide sialique p. 100 mg protéines totales
Sérum	0,99±0,13	2,05±0,32	105 ±3,5	2,0 ±0,11
Exsudat . . .	0,78±0,04	2,42±0,43	73,4±4,8	2,23±0,33

En comparant le diagramme d'électrophorèse sur papier (tampon véronal pH 8,6) de l'exsudat et du sérum du même animal, on constate le plus souvent une diminution très nette de l'intensité de la tache des β- et γ-globulines dans l'exsudat (Figure). La région des α-globulines est difficile à comparer car les exsudats sont le plus souvent hémolysées. On observe alors une bande forte correspondant au complexe Hb-Hp.

Barbara Robert, L. Robert et M. F. Jayle
avec la collaboration technique de
Mlle Monique de Moncuit

Service de Biochimie Médicale, Faculté de Médecine, Paris, le 11 juin 1959.

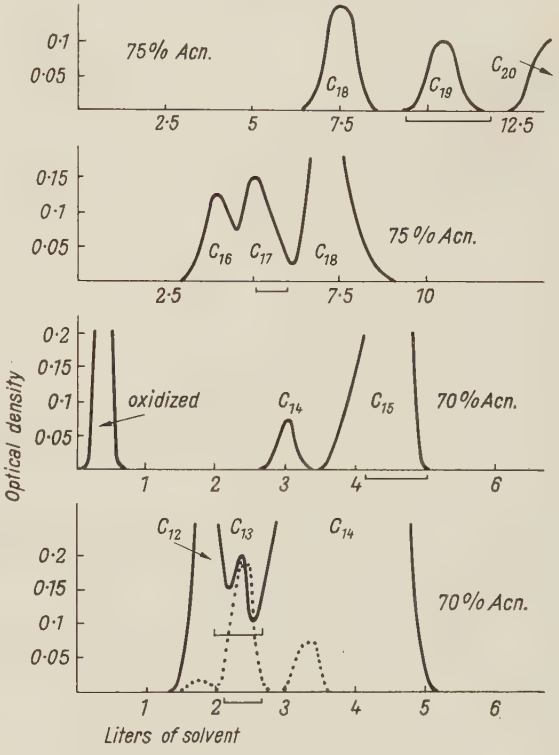
Summary

We studied serum glycoproteins in rats and guinea pigs during the development of granulomas, provoked by carrageenin injection. In rats total serum sialic acid was raised as well as sialic acid soluble in sulfosalicylic acid and haptoglobin. The rise of these 2 fractions, seromucoid and haptoglobine accounts quantitatively for the rise of the total sialic acid. In guinea pigs total serum sialic acid was unchanged, but sulfosalicylosoluble sialic acid as well as haptoglobin were much higher than basal values. Quantitatively the serum glycoprotein reaction was much higher in rats than in guinea pigs.

Column Chromatography of Lipides: Odd-Numbered Straight-Chain Fatty Acids of Menhaden Oil[1]

The occurrence of *n*-nona-, *n*-hepta-, and *n*-pentadecanoic acids in menhaden oil fatty acids had been indicated by microanalytical methods but positive identification was lacking[2,3]. The isolation of these acids and of *n*-tridecanoic acid from menhaden oil is reported here. The essential step after an initial distillation is partition in a reversed-phase column. A novel procedure was developed which enlarges to a preparative scale, the paper chromatographic methods described earlier for the separation of lipides[2,4].

Menhaden fatty acid methyl esters (637 g) were distilled and the fractions analyzed by paper chromatography (acetic and peracetic acid-silicone systems at 30°C and 2°C) or by column chromatography (similar to that described below). Aliquots of the fractions containing saturated normal C_{19}, C_{17} and C_{13} esters were hydrogenated before separation on the column. The fraction rich in C_{15} ester contained *n*-pentadecanoate as the only straight-chain ester of this chain length, together with some myristate and a considerable amount of unsaturated C_{16} esters. Therefore an aliquot of this fraction was oxidized with peracetic acid to facilitate the isolation of the saturated components[4,5].



The separation of methyl esters of saturated fatty acids in a column of silicone on Celite and aqueous acetonitrile (Acn.)

A column, 2·6 cm in diameter and 90 cm in length, was packed with 280 g of a mixture (1:1) of silicone (Dow Corning 200,10 c. s.) and Celite (Johns-Manville). The mobile phase was 70 or 75 % aqueous acetonitrile. The separations represented in the Figure were performed at 25°C with one packing; sample sizes were, in the order of decreasing chain lengths, 0·6, 0·85, 1·4, and 3·9 g of esters. A few milliliters of effluent fractions were diluted with equal volumes of water and the presence of esters was determined under standardized conditions in a spectrophotometer (extinction turbidimetry). A plot of optical density against volume of effluent gave the curves shown in the Figure. Esters located in this manner were chromatographed on paper for tentative identification. The

[1] This work has been supported by a research grant from the National Institutes of Health (USPHS RG 4226) and by the Hormel Foundation.

[2] H. Schlenk, J. L. Gellerman, J. A. Tillotson, and H. K. Mangold, J. Amer. Oil Chem. Soc. *34*, 377 (1957).

[3] R. K. Beerthuis, G. Dijkstra, J. G. Keppler, and J. H. Recourt, Ann. N. Y. Acad. Sci. *72*, 616 (1959).

[4] H. K. Mangold, J. L. Gellerman, and H. Schlenk, Fed. Proc. *17*, 268 (1958).

[5] D. Swern and C. H. Dickel, J. Amer. chem. Soc. *76*, 1957 (1954).

relatively small amount of tridecanoate necessitated rechromatographing on the column.

Suitable fractions were pooled and the esters recovered. The crude materials were subjected to alembic distillations and saponified. Straight-chain odd-numbered acids were still contaminated with branched-chain acids. The straight and branched components superimpose in paper chromatography at room temperature but can be separated by developing the chromatograms at low temperature[2]. By this procedure, we found that *n*-C_{19}, C_{17}, C_{15}, and C_{13} acids occur in menhaden oil to the extent of at least 0·09, 0·65, 0·6, and 0·05% respectively.

The acids were recrystallized two or three times from acetone, and then identified by melting points, mixed melting points, and the long spacings of their crystals. Gas-liquid chromatography showed their purity to be better than 99%. All data confirmed the identity as the normal nona-, hepta-, penta-, and tridecanoic acids.

It has been found previously that C_{16} chain length represents the greatest share of the even-numbered acids in menhaden oil[2]. It is noteworthy that the maximum amount of odd-numbered acids is found with C_{15} and C_{17}. This is in accord with the concept that the latter arise from propionic acid entering into the early phase of chain formation, which then proceeds as with the even-numbered acids[6,7]. The relative amounts of other odd acids, however, are lower than those of the adjacent even acids.

Acknowledgment. The authors wish to thank Dr. L. P. Witnauer for X-ray investigation and Dr. R. T. Holman for gas-liquid chromatography of the samples.

Joanne L. Gellerman and H. Schlenk

The Hormel Institute, University of Minnesota, April 23, 1959.

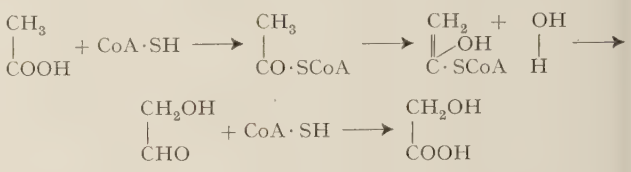
Zusammenfassung

Das Vorkommen von *n*-Nona-, *n*-Hepta-, *n*-Penta- und *n*-Tridekansäure in Menhaden-Öl wird papierchromatographisch bewiesen. Die Säuren werden nach Hydrierung oder Oxydation geeigneter Fraktionen mit Hilfe der Säulenchromatographie isoliert, eine Trennung, die auf der Verteilung der Methylester zwischen Silikon-Öl und wässerigem Acetonitril beruht. Es werden die Anteile der gerad- und ungeradzahligen Säuren des Menhaden-Öles verglichen.

[6] K. El-Shazly, Biochem. J. *51*, 647 (1952).
[7] L. Hartman, J. Amer. Oil Chem. Soc. *34*, 129 (1957).

Probable Mechanism of the Oxidation of Acetate to Glycolate by the Way of the Glycolaldehyde

The possible oxidation of acetate to glycolate has been shown by Challenger *et al.*[1] and by Bernhauer and Scheuer[2], who have obtained glycolate as one of the products of the action of *Asp. niger* on acetate. Moreover, Weinhouse[3] using the same mold, allowed it to metabolize labeled acetate in the presence of unlabeled glycolate has found that appreciable radioactivity was incorporated in the isolated glycolate. The same result has been obtained by us in similar experiments with yeast cells[4]. For the interest in the reaction that leads from acetate to glycolate for our monocarboxylic acid system (MAS) of respiration of acetate[4], we have studied the possible mechanism by which the reaction may occur. As a working hypothesis, we have considered that acetate is activated by the acetate-activating enzyme and that the resulting acetyl-coenzyme A may exist in solution as an equilibrium of the two tautomeric forms[5–8]. By hydration of the enolic form, glycolaldehyde is formed and it is subsequently oxidized to glycolic acid:



If our formulation were true, glycolaldehyde, in the form of its osazone, should have been trapped in experiments of the oxidation of acetate, in the presence of excess phenylhydrazine as a trapping agent. In effect, from these experiments, which were resumed in the present note, glyoxal was isolated in appreciable amounts. The result seems to demonstrate that glycolaldehyde is an intermediate of the oxidation of acetate to glycolate and is therefore also an intermediate of the MAS[4].

Another proof that supports this conclusion, is the fact that from the oxidation of glycolaldehyde by the yeast cells, in presence of phenylhydrazine, the intermediates of the MAS have been obtained, i.e. glyoxylate, formaldehyde, and the formyl group. The doubt that glycolaldehyde might arise from other intermediates of the MAS (glycolate or glyoxylate) is to be excluded, since in submitting these substrates to the oxidation by yeast cells, no glycolaldehyde (glyoxal osazone) could be isolated.

Details for the procedures employed for the experiments with acetate and glycolate[9] or glyoxylate[10] have been described in previous papers. In some experiments the yeast cells were starved by aeration in the presence of 2,4-dinitrophenol before use. The oxidation of glycolaldehyde (Fluka and California Corporation) in presence of phenylhydrazine occurred with the same procedure as was used for glyoxylate[10]. Upon incubation for 8–10 h the yeast cell suspension was centrifuged and in the clear liquid glycolaldehyde was isolated according to the following method. The liquid was concentrated to 3:1 under vacuum, made alkaline to pH 9 and continuously extracted with ether for 12 h. After evaporation of the ether, the extract was dissolved in 25 ml of ethyl alcohol and the alcoholic solution was poured with stirring into 150–200 ml of hot 1% 2,4-dinitrophenylhydrazine (2,4-DP) in 5 *N* H_2SO_4. After boiling under reflux for 30 min all the trap-

[1] F. Challenger, V. Subramaniam, and T. K. Walker, J. chem. Soc. *1927*, 200.
[2] K. Bernhauer and S. Scheuer, Biochem. Z. *253*, 11 (1932).
[3] S. Weinhouse, *Phosphorus Metabolism*, Vol. I (Johns Hopkins Univ. Press, Baltimore 1951), p. 282.
[4] V. Bolcato, B. de Bernard, and G. Leggiero, Arch. Biochem. Biophys. *69*, 372 (1957).
[5] S. Weinhouse, Arch. Biochem. Biophys. *37*, 239 (1952).
[6] S. Ochoa, Adv. Enzymol. *15*, 183 (1954).
[7] M. W. Cronyn, M. P. Chang, and R. A. Wall, J. Amer. chem. Soc. *77*, 3031 (1955).
[8] F. Lynen, Fed. Proc. *12*, 683 (1953).
[9] V. Bolcato, Il Farmaco, Ed. sci. *11*, 431 (1956).
[10] V. Bolcato, Exper. *15*, 222 (1959).
[11] V. Bolcato, Ant. van Leeuwenhoek, in press (1959).

ped phenylhydrazones or osazones were transformed in the corresponding 2,4-DP derivatives. The mixture was allowed to stand for some hours, then filtered, washed, and dried. The material was treated with boiling benzene, filtered, dried, and dissolved in 4 ml of nitrobenzene. To the solution 20 ml of a mixture of benzene, containing 25% of benzine (90–100°C), were added. After filtration, the solution was chromatographed in a Brockmann's alumina column. Development of the chromatogram was carried out with a mixture containing 67·5% of benzene, 22·5% of benzine, and 10% of nitrobenzene. Some red products remained on top of the column, the glyoxal 2,4-DP-osazone in the central portion, and methylglyoxal 2,4-DP osazone in the lower portion. The methylglyoxal arose from the triose phosphates of the endogenous respiration[11]. It was always present in all experiments in which the yeast cells used were not treated with 2,4-dinitrophenol. The glyoxal osazone was eluted with acetone and after partial evaporation of the solvent, it was precipitated with ethyl alcohol. The product melted at 325°C and no depression was observed on admixture with synthetic glyoxal-2,4-DP osazone. Amounts found, mg 20–40/l. Failure to trap larger amounts of glycolaldehyde depended on the fact that it is oxidizable also in the presence of phenylhydrazine, as mentioned above (see also[10]).

V. Bolcato and G. Leggiero

Istituto di Chimica Farmaceutica e Tossicologica dell'Università di Pavia, June 16, 1959.

Riassunto

Dall'ossidazione dell'acetato con cellule intatte di lievito, in presenza di un eccesso di fenilidrazina, è stato isolato l'osazone del gliossale, che rappresenta probabilmente l'aldeide glicolica. Si ritiene perciô che l'aldeide sia un intermedio dell'ossidazione dell'acetato a glicolato secondo la formulazione riportata nel testo.

Catecholamines and Histamine in Vascular Tissue of Normal and Depancreatised Dogs

Since the observations of von Euler[1], the presence of noradrenaline was demonstrated in different tissues, thus also in the walls of the blood vessels[2]. Special significance was attributed to this substance in the arteries by Burn and Rand[3], who have found that an artery deprived of its noradrenaline content by pretreating the animal with reserpine does not react in the usual way to vasoconstricting stimuli. Raab[4] supposes that the adrenaline content of the arteries is in some way connected with atherogenesis.

It seemed of interest to study the adrenaline and noradrenaline concentration in the arteries of normal and diabetic dogs, since the occurrence of late and even early vascular changes in human diabetes mellitus is well known[5].

The dogs were bled in morphine-chloralose narcosis through the carotid artery and segments of different arteries were excised. After washing with saline, drying on filter paper and weighing, the tissues were ground with quartz-sand and trichloracetic acid containing ascorbic acid. The suspension was centrifuged and the catecholamines were chromatographed on aluminium oxid. After washing, the catecholamines were eluted with acetic acid and the Euler-Floding reaction was performed. The reaction is specific for adrenaline at pH 3·45, while both catecholamines give the reaction at pH 5·45 as described previously[6]. The results are expressed in μg/g wet weight.

The results in normal dogs are summarised in Table I. With the exception of the femoral artery, the sum of the two catecholamines is 1·0–1·5 μg/g, adrenaline representing only 5–6% of the total.

Totally depancreatised dogs were maintained for 27–55 days on insulin. The treatment was abandoned three days prior to the experiment. The results are given in Table II. It is evident that, with the exception of the femoral artery, the adrenaline concentration increased significantly compared with the vessels of normal dogs ($p < 0{\cdot}05$). The noradrenaline concentration showed a decrease, but according to statistical analysis this decrease did not prove significant ($p > 0{\cdot}05$). The sum of the concentration of the two catecholamines showed a small decrease too. The most significant finding is the change in the percentage of the adrenaline fraction which proved to be 10–35% in

[1] U. S. von Euler, Acta physiol. Scand. *12*, 73 (1946).

[2] C. G. Schmiterlöw, Acta physiol. Scand. *16*, Suppl. 56 (1948). – U. S. von Euler and F. Lishajko, Acta physiol. Scand. *42*, 333 (1958). – W. Raab and W. Gigee, Angiology *9*, 283 (1958).

[3] J. H. Burn and M. J. Rand, Brit. med. J. *1*, 903 (1958).

[4] W. Raab, Amer. J. Cardiology *1*, 113 (1958).

[5] F. R. Bárány, *Abnormal vascular reactions in diabetes mellitus* (Lund 1955).

[6] I. Faredin and B. Sárkány, Kisérletes Orvostudomány *10*, 174 (1958).

Table I

	Number of dogs	Adrenaline (A)	Noradrenaline (NA)	Sum of A+NA	Percentage of A
Femoral artery	10	0·039 ± 0·005	0·470 ± 0·072	0·509 ± 0·077	7·6
Carotid artery	12	0·056 ± 0·006	1·112 ± 0·129	1·168 ± 0·135	4·8
Renal artery	11	0·073 ± 0·007	1·445 ± 0·221	1·518 ± 0·228	4·8
Coeliac artery	7	0·075 ± 0·011	1·440 ± 0·284	1·515 ± 0·295	4·9
Abdominal aorta	9	0·093 ± 0·015	1·368 ± 0·305	1·461 ± 0·320	6·3

Mean adrenaline and noradrenaline concentrations expressed in μg/g tissue with standard deviations, in normal dogs.

Table II

	Number of dogs	Adrenaline (A)	Noradrenaline (NA)	Sum of A+NA	Percentage of A
Femoral artery	9	0·053 ± 0·007	0·307 ± 0·067	0·360 ± 0·074	14·7
Carotid artery	12	0·102 ± 0·022	0·890 ± 0·145	0·992 ± 0·167	10·2
Renal artery	12	0·191 ± 0·042	0·986 ± 0·214	1·177 ± 0·256	16·2
Coeliac artery	9	0·225 ± 0·045	0·697 ± 0·183	0·922 ± 0·228	24·5
Abdominal aorta	10	0·317 ± 0·060	0·602 ± 0·224	0·919 ± 0·284	34·6

Corresponding data on diabetic dogs, 27–55 days after pancreatectomy.

Table III

	Number of dogs	Adrenaline (A)	Noradrenaline (NA)	Sum of A+NA	Percentage of A
Femoral artery	4	0·045 ± 0·017	0·464 ± 0·030	0·509 ± 0·047	8·9
Carotid artery	4	0·058 ± 0·019	0·735 ± 0·234	0·793 ± 0·253	7·3
Renal artery	3	0·139 ± 0·039	1·718 ± 0·300	1·857 ± 0·339	7·5
Coeliac artery	4	0·095 ± 0·024	1·704 ± 0·204	1·799 ± 0·228	5·3
Abdominal aorta	3	0·169 ± 0·040	1·194 ± 0·565	1·363 ± 0·605	12·4

Corresponding data on diabetic dogs, 7–9 days after pancreatectomy.

Table IV

	Normal dogs (8)	Diabetic dogs 7–9 days after pancreatectomy (2)	Diabetic dogs 27–55 days after pancreatectomy (10)
Femoral artery	1·3 ± 0·2	0·9	0·9 ± 0·1
Carotid artery	1·1 ± 0·3	1·3	0·9 ± 0·1
Renal artery	4·2 ± 1·2	5·6	3·9 ± 0·6
Coeliac artery	6·3 ± 1·2	–	3·2 ± 0·4
Abdominal aorta	2·9 ± 0·7	2·6	3·1 ± 0·7

Mean histamine concentrations expressed in μg/g tissue with standard deviations. Number of animals in each group in parenthesis.

the different vessels of the pancreatectomised dogs. These values reached only 5–7% in normal dogs.

Pancreatectomy *per se* does not lead to similar alterations: only slight changes were found in the adrenaline concentration of the arteries of diabetic dogs killed 7–9 days after the operation (Table III).

For the determination of histamine—due to the presence of substances causing relaxation of the guinea pig ileum—some inadequacies were observed with the classic Barsoum-Gaddum-Code-Method. Therefore, before the biological test was performed, an extract was made with an ionic exchange resin[7]. The average concentration of histamine in the vessel walls was found to be 1–6 μg/g, no significant difference occurring between the three groups of animals (Table IV).

The importance of the above findings is as yet not clear. Whether adrenaline plays any rôle in atherogenesis is open to debate. According to Raab[2], adrenaline may promote the fatty infiltration of the intima by causing local hypoxia. Its administration intensifies the deposition of cholesterol in the intimal tissue; catecholamine releasing mechanisms produce or aggravate vascular lesions. Although the physiological significance of the constancy of the adrenaline-noradrenaline ratio is as yet not well known, the biochemical abnormality described above may be in some correlation with the development of functional derangements and, after long standing, probably even with morphological changes of the blood vessels.

I. Faredin, M. Winter, B. Taños, and G. HeténYi†

First Department of Medicine, University Medical School, Szeged (Hungary), April 20, 1959.

Zusammenfassung

Der Adrenalin-, Noradrenalin- und Histamingehalt wurde in verschiedenen Arterien normaler und pankreaektomierter Hunde bestimmt. Die Konzentration von Adrenalin nahm in den Gefässen der seit 27–55 Tagen diabetischen Tieren zu, die von Noradrenalin mässig ab. Der Histamingehalt blieb unverändert. Der Anteil von Adrenalin am gesamten Katecholamingehalt der Gefässe war 5–6% in normalen, 10–35% in diabetischen Hunden. Es wird auf die Möglichkeit hingewiesen, dass die beschriebenen Veränderungen mit den diabetischen Gefässleiden im Zusammenhang stehen.

[7] I. Faredin, J. Borbola, and G. Bikich, Acta med. Acad. Sci. hung. *6*, 195 (1954).

Behavior of Capillary Endothelium During Dextran Induced Oedema

Systemic administration of anaphylactoid substances like dextran or eggwhite induces oedema in the rat[1]. Alterations in the capillary bed during oedema formation were investigated in our laboratories and observations are reported in this communication.

Materials and Methods.—Eight-week-old Sprague-Dawley albino rats were kept on standard diet. The dilute India ink contained 3% carbon and 3% gelatine. Each rat received 0·5 ml/100 g intravenously. The acid dye colloid was a 0·4% Trypan blue solution, of which animals received 0·5 ml/100 g body weight intravenously. India ink in blood was determined as by HELLER *et al.*[2]. Amount of carbon in blood is expressed as μg/ml.

Molecular weight of dextran was 82,600. Fresh eggs were used for obtaining eggwhite, which was *ana partes* diluted and homogenized. Compounds provoking colloid storage were injected intradermally in 0·1 ml volume and readings were made on the inner surface of the removed abdominal skin. Histamine hydrochloride was used and compound 48/80 (a polymer of N-methyl-homoanisylamine and formaldehyd) kindly donated by Dr. E. J. DE BEER from the Welcome laboratories.

Results.—For each rat 30 mg dextran was injected intraperitoneally. India ink was injected at different time intervals after dextran; before and during the oedema formation and as the oedema fully developed. Simultaneously with India ink, each animal received 10 μg histamine and 10 μg/48/80 intradermally into the abdominal skin. The rate of India ink clearance from the blood stream was determined every 10 min after the injection of India ink. Animals were killed 2–3 h after dextran administration and India ink accumulation was registered as to ++++ as estimated at the feet, face, and ears as well as on the abdominal skin where histamine and 48/80 was injected.

The Table shows that no oedema developed 30 min after dextran and, at the same time, an intense India ink accumulation is present at the sites where later oedema developed and where histamine and 48/80 were injected. During the presence of intense dextran-induced oedema, no India ink accumulates at the extremities and where stimuli (histamine, 48/80) were applied. As the oedema progressively diminished (180 min) and disappeared (240 min), the India ink accumulation reappeared again with increasing intensity. The rate of India ink clearance is normal as oedema is absent but considerably delayed during the presence of oedema at 60 and 90 min after dextran administration.

The same phenomena were observed if oedema was induced with eggwhite, another anaphylactoid substance, and if Trypan blue was injected instead of India ink. During the presence of eggwhite-induced oedema, there is no dye accumulation on the capillary endothelium and there is a delayed rate of India ink clearance during the same period of time.

Discussion.—Systemic administration of anaphylactoid substances produces characteristic syndromes in rats:

(1) A transitory vasodepression, caused by the liberated histamine. This vasodepression lasts for a few minutes after the injection of the anaphylactoid substances[1,3,4].

(2) Oedema formation at the sites of predilection[1].

(3) Haemoconcentration during the presence of oedema, reaching a peak simultaneously with the maximum intensity of oedema[1,4].

(4) Hypothermia, which follows a parallel curve with the haemoconcentration[1].

(5) In the surviving tissues of rats treated with anaphylactoid substances, increased amounts of histamine were found[5].

To this syndrome of the anaphylactoid reaction further characteristic alterations can be added as results from our experiments.

(1) India ink accumulates at the extremities where later oedema develops as well as in the skin where stimuli (histamine or 48/80) are used, before the oedema appears.

(2) There is no endothelial stickiness at the sites of oedema or at any other sites of the skin during the presence of anaphylactoid oedema. No India ink accumulation can be seen at the extremities and no accumulation can be provoked by histamine or 48/80 as long as the oedema lasts.

[1] J. H. HELLER, R. M. MEIER, R. ZUCKER, and G. W. MAST, Endocrinology *61*, 235 (1957).

[2] M. BRIOT and B. N. HALPERN, C. R. Soc. biol., Paris *146*, 15 (1952).

[3] B. N. HALPERN and M. BRIOT, C. R. Soc. biol., Paris *148*, 1046 (1954).

[4] C. H. HANNA and L. H. MARSHALL, Amer. J. Physiol. *191*, 615 (1957).

[5] B. N. HALPERN and M. BRIOT, C. R. Soc. biol., Paris *143*, 1552 (1952).

			India ink intravenously min after dextran				
			30	60	90	180	240
Intensity of oedema			–	++++	++++	++	–
India ink accumulation	Histamine		++++	±	–	++	++++
	48/80		++++	+	–	++	++++
	Feet		++++	–	–	++	++++
	Face		+++	±	–	+	+++
	Ears		+++	+	–	±	+++
Carbon content in blood μg/ml	min after India ink	15	236	1085	960	325	264
		25	123	719	780	272	192
		35	108	535	355	233	119
		45	74	207	216	181	97

Intensity of oedema at different time intervals after 30 mg dextran intraperitoneally India ink accumulation in the skin of the extremities and where histamine and 48/80 was injected. Carbon content in blood as measured 15, 25, 35, and 45 min after India ink was injected. Each mark represents average readings on ten rats.

(3) During anaphylactoid oedema there is a delayed carbon particle clearance from the blood stream.

At the present time no interpretation of the results obtained can be attempted. The altered systemic capillary response during anaphylactoid oedema is an intriguing fact and merits further consideration.

B. Gözsy and L. Kátó

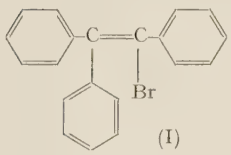
Institute of Microbiology and Hygiene of the University of Montreal (Canada), March 17, 1959.

Résumé

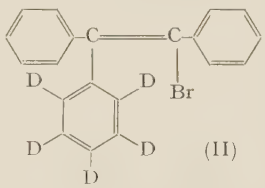
Durant la présence de l'œdème, provoqué par le dextran, l'endothélium capillaire perd, partout dans la peau du rat, la capacité de devenir adhésif après un stimulus. Ce comportement réfractaire des cellules endothéliales est reversible. Pendant la même période, la disparition de l'encre de Chine injectée dans le sang est considérablement retardée.

Différence d'activité oestrogène entre l'α-bromotriphényléthylène et un de ses analogues pentadeutérié

Il a déjà été constaté que les propriétés biologiques des molécules organiques deutériées ne sont pas toujours identiques à celles de leurs analogues contenant de l'hydrogène[1]. Dans le cadre de ce même type d'études, nous avons recherché s'il existe une différence d'activité œstrogène entre l'α-bromotriphényléthylène normal (I) et un de ses analogues qui est assez riche en deutérium: l'α-bromo-α,β-diphényl-β-pentadeutériophényléthylène (II); la synthèse de ce dernier composé a été décrite récemment[2].



(I)



(II)

Nous utilisons le test d'Allen-Doisy sur la rate castrée, prenant comme critère la réapparition du cycle œstral chez le castrat, constatée par l'étude des frottis vaginaux. Nous avons vérifié au préalable la sensibilité à la folliculine des rates castrées utilisées, et les produits sont injectés par voie sous-cutanée en solution huileuse (0,2 cm³). La zône de sensibilité propre à l'α-bromotriphényléthylène chez ces castrats se situe entre 0,75 et 5 γ selon les individus.

Chaque lot de rates est traité une fois tous les 15 jours avec la dose correspondant à sa sensibilité, de façon à provoquer autant que possible une proportion d'œstrus comprise entre 10 et 90%. Les frottis sont exécutés aux temps suivants: 0, 24, 42, 48, 66, 72, 90 et 96 h.

Etant donné que l'on se trouve dans une zône sensible comprise entre 0 et 100% d'œstrus, les réactions observées se présentaient souvent comme un début de cycle, celui-ci s'arrêtant au préœstrus sans aller jusqu'aux cellules kératinisées pures. Aussi, avons-nous trouvé bon d'exprimer également les résultats par les nombres des préœstrus obtenus avec les deux produits.

Enfin, nous avons noté la durée de l'œstrus, et l'avons chiffré arbitrairement en totalisant, dans chaque lot et sur l'étendue des 96 h d'observation, le nombre de frottis présentant un œstrus franc. Nous avons donc ainsi obtenu trois séries de chiffres, contenus dans les tableaux ci-dessous. Il est à noter que la différence entre les poids moléculaires des deux composés (335 pour (I) contre 340 pour (II)) est extrêmement faible et peut être considérée ici comme de second ordre.

Tableau I

Pourcentage d'œstrus

Dose (en γ)	Nombre d'œstrus avec la substance (I)*	Nombre d'œstrus avec la substance (II)*
10	6/6 rates	6/6 rates
5	16/20	8/24
1	5/6	2/6
0,75	7/24	3/24
	Totaux 34/56	19/60

* Le premier chiffre représente le nombre de rates présentant un œstrus, le deuxième représente le nombre de rates ayant reçu la substance.

Tableau II

Pourcentage total de préœstrus

Doses (en γ)	Nombre de préœstrus avec la substance (I)*	Nombre de préœstrus avec la substance (II)*
10, 5, 1 et 0,75	47/56	50/60

* Le premier chiffre représente le nombre de rates présentant un préœstrus, le deuxième représente le nombre de rates ayant reçu la substance.

Tableau III

Durée d'œstrus (chiffrée arbitrairement)

Dose (en γ)	Substance (I)	Substance (II)
10	26	25
1	15	2
5	6	0
0,75	5	1
5	15	20
0,75	3	0
5	16	11
0,75	9	4
5	12	0
0,75	1	3
	Totaux 108	66

Conclusions. Au point de vue œstrus (nombre et durée), il y a un écart très net entre les deux séries, le composé (I) étant le plus actif. Cet écart ne se retrouve pas avec les préœstrus, sur lesquels les chiffres sont à égalité, ce qui doit signifier que l'α-bromo-α,β-diphényl-β-pentadeutériophényléthylène n'arrive généralement pas, aux doses

[1] A. Lacassagne, N. P. Buu-Hoï, A. Chamorro, N. D. Xuong et N. Hoán, C. R. Acad. Sci. Paris *231*, 1384 (1950). – N. P. Buu-Hoï et N. D. Xuong, Communication au 2e Congrès international sur l'Utilisation pacifique de l'Energie atomique (Genève) *25*, 223 (1958).

[2] N. P. Buu-Hoï et N. D. Xuong, C. R. Acad. Sci. Paris *247*, 654 (1958).

essayées, à achever son activité œstrogène en allant jusqu'à l'œstrus. Ces résultats s'expliquent soit par les différences stériques qui existent entre la molécule d'œstrogène deutériée et celle de son analogue, soit par la présence d'un effet isotopique dans la métabolisation de la molécule (II) (par exemple dans l'hydroxylation en position *para*).

N. P. Buu-Hoi, N. D. Xuong et A. Beauvillain

Institut du Radium de l'Université de Paris, le 20 avril 1959.

Summary

The oestrogenic activity of α-bromo-α,β-diphenyl-β-pentadeuteriophenylethylene is compared with that of the non-deuterated analogue, and found to be significantly lower. No sizeable difference was found between the two compounds as regards their preoestrus-inducing activity.

Turbidimetric Detection of Decamethonium

The presence of very low amounts of decamethonium (C_{10}) salts is usually detected by biological tests or by radioactivity measurements[1] of C^{14} labelled C_{10}. The biological methods, even very sensitive, offer some disadvantage for accurate quantitative estimation. The isotopic methods give exact results, but are independent from the degradation state of the C_{10} molecule.

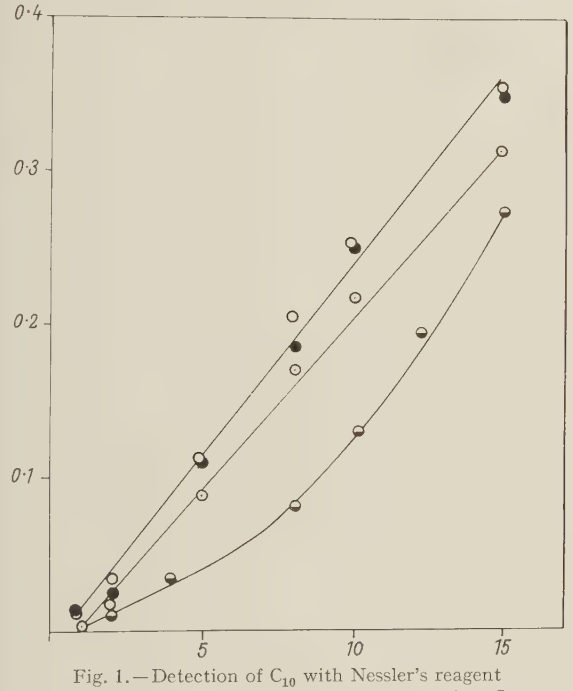


Fig. 1.—Detection of C_{10} with Nessler's reagent
On the ordinate optical density values; on the abscissa C_{10} concentrations expressed in γ/ml

water ⊙—⊙ 0·5 *M* NaCl
1 *M* NaCl ○—○ 2 *M* NaCl

Zaimis[2] has proposed a method based on the reaction of methonium salts with Reinecke salt, which unfortunately is not applicable to C_{10} detection in biological fluids, for the fact that the minimal amount (10 mg) required for reaction is greater than that usually employed as myorelaxant.

We present here a turbidimetric method for C_{10} determination useful for concentrations between 2 and 20 γ/ml.

A turbidity develops a few minutes after the addition of 0·1 ml of Nessler's reagent to 2·9 ml of a solution containing C_{10}. Readings are then taken after $^1/_2$ h, using a Beckman DU spectrophotometer at 580 mμ against a blank containing the same quantity of Nessler's reagent. When the turbidities for crescent quantities of C_{10} are plotted, there is a complete linear relationship, if the reaction occurs in solution of 1 *M* NaCl or at higher concentrations, and this depends on the electrostatic nature of the complex. Figure 1 shows the reactions of crescent quantities of C_{10} in water and at different NaCl molarities.

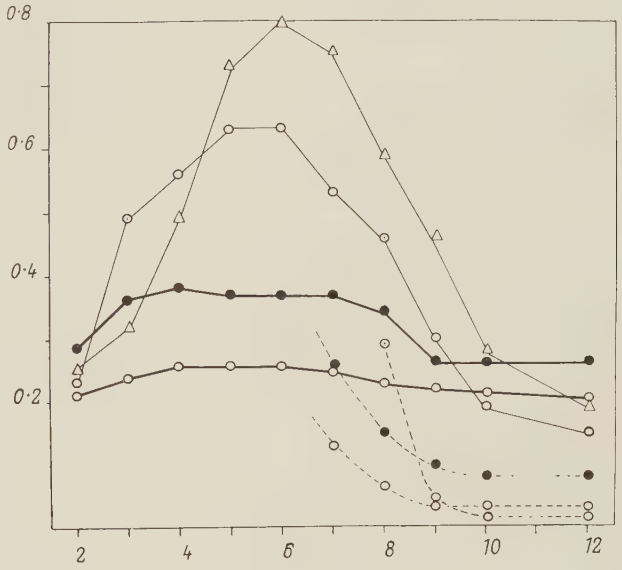


Fig. 2. pH influence on turbidity developed by some anesthetic drugs. On the ordinate optical density values; on the abscissa pH values. Concentration 10 γ/ml for all substances

C_{10} in water ○—○ C_{10} in 2 M NaCl
⊙—⊙ C_6 ▽—▽ C_5 ⊙- - -⊙ Decamethylendiamine
●· · ·● Fargan ○ · · ○ Lobeline

A large number of substances that are used in anesthetic practice was tested in order to investigate the specificity of the reaction. At acid or neutral pH values, only a few have developed a turbidity, which in alkali becomes negligible. It was proved that in alkali the reaction is absolutely specific for methonium salts. Figure 2 shows the influence of pH on methonium salt reactions and on anesthetic drug reactions.

The reaction was therefore effected in 2 *M* NaCl, without buffers, at pH values (10–13) given directly to solution by addition of the strongly alkaline Nessler's reagent.

The relation between the length of the carbon chain and the development of turbidity has been checked by testing pentamethonium (C_5) and hexamethonium (C_6). Figure 3 shows that the turbidity is roughly proportional to carbon chain length.

The effect of quarternary ammonium groups was checked by testing decamethylendiamine. No appreciable turbidity occurs for this compound, as expected by the lack of positively charged ammonium groups. The methylen carbon chain is also essential for turbidity devel-

[1] C. Manni, G. Moricca, B. Giovanella, and P. Mazzoni, Simposio Internazionale su Curaro, Curarosimili e Curarizzanti (I.T.E. 1958), p. 240.

[2] E. Zaimis, Brit. J. Pharmacol. *5*, 424 (1950).

opment as demonstrated by the complete lack of turbidity for succinylcholine.

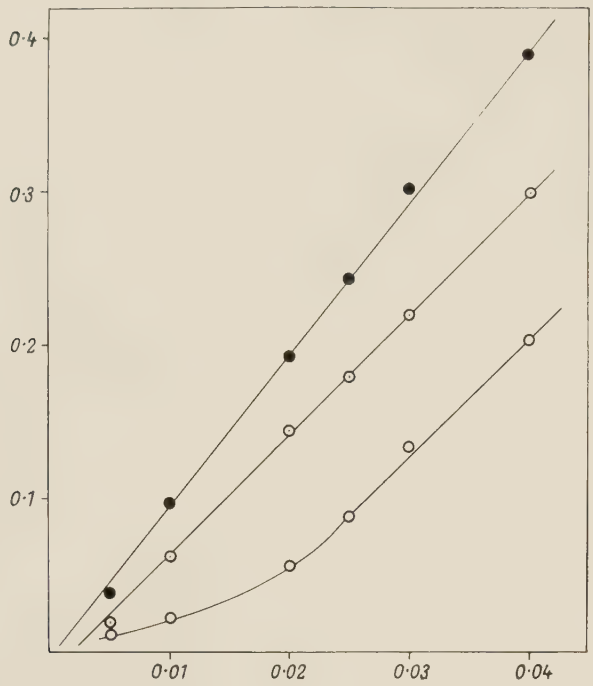


Fig. 3.—Influence of carbon chain length on the reaction of methonium salts with Nessler's reagent. On the ordinate optical density values; on the abscissa methonium salt concentrations expressed in μM/ml.

● —● C_{10} ⊖—⊖ C_6 ○—○ C_5

The application of the described method for the detection of C_{10} in biological fluids, and the results obtained in experimental research as in clinical investigations will be fully reported elsewhere.

B. Giovanella, C. Manni, and G. Moricca

Istituto Regina Elena per lo Studio e la Cura dei Tumori, Roma, and Clinica Chirurgica, Università di Roma, June 10, 1959.

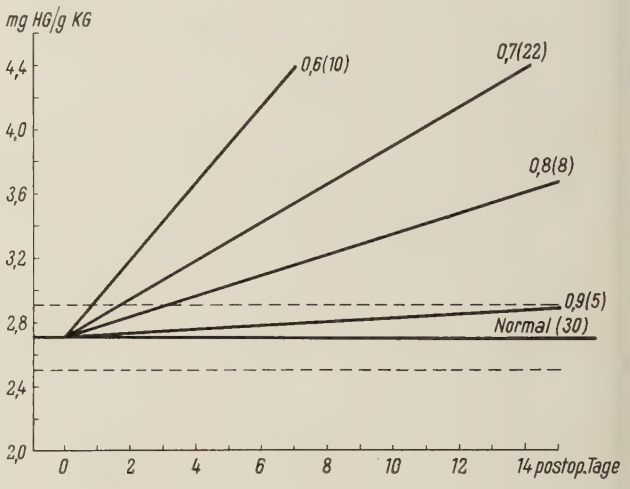
Riassunto

Si descrive un metodo per la determinazione del decametonio in piccole concentrazioni (2–20 γ/ml) impiegando il reattivo di Nessler.

Si sono identificate le condizioni in cui la reazione torbidimetrica che si produce è strettamente specifica, e può quindi essere utilmente impiegata per il dosaggio di questo miorilassante nei liquidi biologici.

Das Verhalten des Glykogens bei linksventrikulärer Hypertrophie nach artifizieller peripherer Widerstandserhöhung

Margaret Beznàk[1] hat in eingehenden physiologischen Studien das Verhalten des Kreislaufes und des Herzens nach stenosierender Beringung der Abdominalaorta bei Ratten abgeklärt. Diese Untersuchungen zeigen, dass im Anschluss an die artifizielle Stenosierung der Aorta der totale periphere Widerstand ansteigt; der prästenotische arterielle Mitteldruck bleibt anfänglich unverändert, um in der Folge langsam anzusteigen; der poststenotische arterielle Mitteldruck fällt postoperativ ab, steigt später ebenfalls langsam an und erreicht schliesslich hypertone Druckwerte. Parallel mit der Entstehung der Hypertonie tritt eine mehr oder weniger ausgesprochene linksventrikuläre Hypertrophie, die 50 und mehr % des Normalgewichtes betragen kann, auf. Im weiteren konnte Beznàk zeigen, dass die Entstehung der Hypertonie wie auch der Hypertrophie von einer funktionstüchtigen Hypophyse abhängt. Nach Hypophysektomie weisen sowohl Normaltiere wie Stenosetiere eine Hypotonie und eine Atrophie des Herzens auf. Diese hämodynamisch gut definierte Form der Hypertrophie des Herzens schien uns zu Stoffwechselversuchen am Kleintier besonders geeignet zu sein.



Entstehung der Herzhypertrophie nach artifizieller Stenosierung *der Abdominalaorta*

In der Ordinate ist ein Quotient (mg Herzgewicht pro 1 g Körpergewicht), der das Ausmass der Hypertrophie wiedergibt, eingezeichnet. Auf der Abszisse befindet sich die postoperativ verstrichene Zeit in Tagen. Die nicht eingeklammerten Zahlen hinter den verschiedenen Steigungslinien geben den innern Durchmesser der verwendeten Goldringe an (0,6, 0,7, 0,8, 0,9). Die eingeklammerten Zahlen geben die zur Errechnung der Linien verwendeten Tiere an

Da, nach Russell und Bloom[2–4], durch ACTH oder Wachstumshormon STH der Glykogengehalt des Herzens erhöht werden kann und andererseits die Entstehung der Hypertonie-Hypertrophie des Herzens von einer funktionstüchtigen Hypophyse abhängt, schien uns eine Kontrolle des Glykogengehaltes beim hypertrophierenden Rattenherz angezeigt. Eine eventuelle Überfunktion der Hypophyse könnte theoretisch zu einer Glykogensteigerung des Herzmuskels führen.

Methodik. Wir verwendeten männliche Albinoratten im Gewicht von 150–210 g. Die Aorta wurde von einer linksseitigen Abdominalöffnung aus direkt unterhalb des Zwerchfelles mit einem Goldring von 0,9, 0,8, 0,7 oder 0,6 mm innerem Durchmesser umgeben. Die Vergleichs-

[1] Margaret beznàk, J. Physiol. *116*, 74 (1952); Canad. J. Biochem. Physiol. *33*, 985, 995 (1955); *34*, 791 (1956); Circ. Res. *6*, 207 (1958).

[2] Jane A. Russell and A. E. Wilhelmi, Endocrinology *47*, 26 (1950).

[3] Anne E. Milman and Jane A. Russell, Endocrinology *47*, 114 (1950).

[4] Jane A. Russell and W. L. Bloom, Amer. J. Physiol. *183*, 345 (1955); Endocrinology *58*, 83 (1956).

Glykogengehalt normaler und hypertrophierender Rattenherzen

	Zahl der Tiere	a Alkalisch lösliches Gesamtglykogen in mg/g Feuchtgewicht	b säurelösliches Glykogen in mg/g Feuchtgewicht	Proteingebundenes Glykogen = Differenz von a–b
Normaltiere . . .	15	4,8 ± 1,1	1,2 ± 0,38	3,6
Ringtiere	24	6,1 ± 1,4	1,9 ± 0,76	4,2
Anstieg des Glykogens in % der Normalwerte . .		27%	58%	16%
P-Faktor		> 0,001 < 0,01	< 0,001	

tiere wurden der gleichen operativen Technik unterzogen, wobei ebenfalls die Aorta subdiaphragmal freipräpariert wurde.

Das säurelösliche und alkalilösliche Glykogen des linksventrikulären Herzmuskels wurden nach sofortiger Gefrierung des Herzens in flüssigem Stickstoff nach den Angaben von Bloom[5] bestimmt.

Resultate. In der Abbildung ist das Verhalten des Gewichtes der beiden Herzkammern zusammen in Abhängigkeit des inneren Durchmessers des Aortenringes und der postoperativ verstrichenen Zeit dargestellt. Als Mass für die Hypertrophie des Herzens wurde ein Quotient (Herzgewicht in mg pro 1 g Körpergewicht) errechnet.

Bei 30 unberingten operierten Tieren ergab sich ein Quotient von 2,7 ± 0,2 mg Herzgewicht pro 1 g Körpergewicht. Die mit einem Ring von 0,6 mm Durchmesser versehenen Tiere erreichen am 7. Tag im Mittel einen solchen von 4,4 (10 Tiere). Bei dieser Gruppe überlebte nur ein kleiner Teil den 7. postoperativen Tag. Etwa $^2/_3$ der Tiere gehen in den ersten 3 Tagen an einer Linksinsuffizienz mit Lungenödem ein; selten einmal können die Zeichen einer globalen Herzinsuffizienz festgestellt werden. Bei 22 Tieren mit einem Ring von 0,7 mm Durchmesser steigt der mittlere Faktor in 14 Tagen auf 4,4. Bei dieser Gruppe stellt eine akute Herzinsuffizienz ein seltenes Ereignis dar. Ist der Ringdurchmesser 0,8 mm, so steigt der Quotient bis zum 15. Tag auf 3,7 (8 Tiere). Beträgt die Ringgrösse 0,9 mm, so fällt der errechnete Quotient am 15. Tag immer noch in die Streubreite der Normaltiere und beträgt im Mittel aus 5 Tieren 2,9. Bei den letzten beiden Gruppen kam postoperativ kein einziges Tier auf Grund einer Herzinsuffizienz *ad exitum*. Die Streubreiten der einzelnen Gruppen sind ziemlich gross, was durch die Schwierigkeit eines vollständigen Ringschlusses bedingt sein dürfte. Die Kontrolle der Trockensubstanz des Herzmuskels pro 1 g Feuchtgewebe ergab in allen Gruppen keine Abweichung von denen der Normaltiere. Dadurch kann eine eventuelle Ödembildung ausgeschlossen werden.

In einer ersten Serie von Tieren, die mit Ringweiten von 0,7 und 0,8 mm versehen waren, prüften wir sowohl die säurelösliche wie auch die alkalilösliche (Gesamt-)-Glykogenfraktion. Die Tiere hungerten vor der Tötung während 24 h. Es zeigte sich dabei, dass sowohl die säurelösliche wie auch die alkalilösliche Fraktion des Glykogens vom 4. postoperativen Tag an bei hypertrophierenden Herzen signifikant erhöht ist (Tabelle). Dabei verteilt sich die Zunahme des Glykogens zu etwa gleichen Teilen auf die säurelösliche wie auf die proteingebundene Fraktion (proteingebundene Fraktion = alkalilösliche minus säurelösliche Fraktion). Der prozentuale Anstieg der alkalilöslichen Fraktion beträgt 27%, derjenige der säurelöslichen Fraktion 58% des Normalwertes. Die Enge des Ringes, das heisst die Geschwindigkeit der Entstehung der Herzhypertrophie, verändert die Glykogenzunahme.

Da über die ersten 3 postoperativen Tage keine einheitlichen Glykogenverhältnisse gefunden werden konnten, versuchten wir durch extremen Hunger wie auch durch kohlehydratreiche Sondenernährung mit nachfolgender 24stündiger Hungerperiode Klarheit zu erlangen. Auch dadurch gelang es nicht, eine statistisch gesicherte Veränderung im Glykogengehalt des Herzens nachzuweisen. Es fiel allerdings auf, dass ungefähr 48 h nach erfolgter Operation sowohl bei beringten wie nicht beringten Tieren ein ausserordentlich starker, kurzdauernder Glykogenanstieg auftrat.

Bei einer letzten Serie von Tieren, die mit der Sonde schon präoperativ wie auch postoperativ kohlenhydratreich ernährt wurden, verabreichten wir 4 h vor der Tötung durch die Sonde 2 g Glukose. Dabei traten schon in den ersten 24 h nach Beringung der Aorta erhöhte Glykogenwerte auf.

Diskussion. Aus unseren Versuchen geht hervor, dass eine enge Beziehung zwischen dem innern Durchmesser des Aortenringes und der gewichtsmässigen Hypertrophie des linken Herzens besteht. Damit bestätigen wir die Feststellung von Beznàk, welche im weiteren zeigte, dass, hämodynamisch gesehen, die Beringung vorwiegend eine periphere Widerstandserhöhung verursacht. Je enger der Aortenring, das heisst je grösser der periphere Widerstand, desto rascher entwickelt sich eine Hypertrophie des Herzens.

Wie unsere Versuche zeigen, finden wir beim hypertrophierenden Rattenherzen einen deutlichen Anstieg sowohl der säurelöslichen wie auch der alkalilöslichen Glykogenfraktion. Dabei scheint die Geschwindigkeit der Hypertrophie-Entstehung einen Einfluss auf die Grösse des Glykogenanstieges auszuüben. Es gelingt auch durch bestimmte Ernährung der Tiere, schon einige Stunden nach der operativen Beringung den Glykogenanstieg nachzuweisen.

Nach den Versuchen von Beznàk steht fest, dass die Entstehung der Hypertonie-Hypertrophie nach peripherer Widerstandserhöhung von einer funktionstüchtigen Hypophyse abhängt. Im weiteren zeigten Bloom und Russell, dass eine Verabreichung von STH oder ACTH den Glykogengehalt des Herzmuskels deutlich erhöht, während Hypophysektomie zu einer Verminderung desselben führt. *Die Überlegung liegt nahe, dass die Erhöhung des Glykogenspiegels im hypertrophierenden Rattenherzen, wie wir sie festgestellt haben, ebenfalls von einer vermehrten Hypophysenaktivität abhängt* und ähnliche Verhältnisse entstehen, wie sie nach Verabreichung von STH oder ACTH zu finden sind. Einen Beweis für diese Annahme

[5] W. L. Bloom, G. T. Lewis, Mary Z. Schumpert, and Tsung-Men Shen, J. biol. Chem. *188*, 631 (1951).

können wir allerdings mit unseren Resultaten nicht geben und möchten auch die Frage offen lassen, ob nicht eine eventuelle postoperative Durchblutungsstörung des Splanchnicusgebietes an der Entstehung des Glykogenanstieges beteiligt ist, da, wie ebenfalls aus Arbeiten von Bloom und Russell hervorgeht, durch Hunger eine Erhöhung des Herzglykogens erzielt wird.

E. Lüthy, B. Bischof und H. Rösli

Medizinische Universitäts-Poliklinik Zürich, 23. Juni 1959.

Summary

By an artificial stenosis of the abdominal aorta, hypertrophy of the left heart is produced. Different degrees of narrowing of the aorta lead to different degrees of heart hypertrophy. After a starvation of 24 h, the glycogen content of the heart that is in a hypertrophic condition is higher than in normal hearts.

Lead Reactive Substances in Peripheral Synapses

The subneural apparatus of motor end-plates was first demonstrated with a supravital Janus-Green B staining by Couteaux[1]. Histochemical investigations revealed that the palisade-like structure of the subneural apparatus exhibits cholinesterase activity[2–8].

Biochemical investigations concerning the function of the myoneural synapsis suggest, however, that a number of other factors play an essential rôle in the transmission of impulses. According to Koshtoyantz *et al.*[9], especially –SH compounds must be taken into account. The participation of active –SH groups in the structure of Coenzyme A, an essential factor in the manufacturing of acetylcholine, is a well-known fact[10]. Considering the very sensitive reaction of heavy metal salts with –SH groups (mercaptide reaction), it was decided to attempt to trace the subneural apparatus and other synaptical structures by means of lead compounds.

Technique and Results. Anesthesia was induced in rats by an intraperitoneal injection of urethane (0·2–0·3 g). One ml of the following solution was injected percutaneously into the short flexor muscle of the hind pad:

$Pb(NO_3)_2$ cryst.	1·0 g
Urea (or guanidine)	1·0 g
Formalin 4 p.c.	20·0 ml

15 min later the animals were killed and the muscles excised. Frozen sections were cut immediately, washed briefly in distilled water and immersed for a few seconds in a 2 p.c. aqueous solution of sodium sulfide. This treatment resulted in the characteristic microscopical pattern of the subneural apparatus, as had been anticipated.

Fig. 1

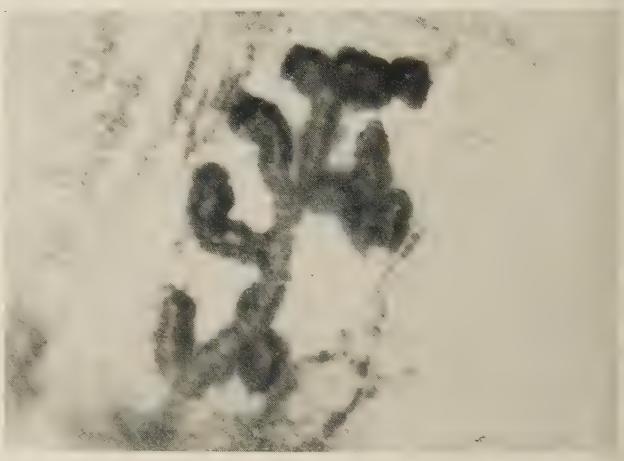


Fig. 2

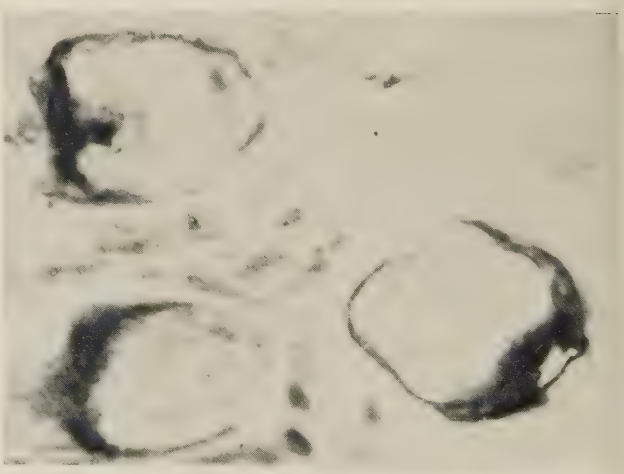


Fig. 3

Similar results were obtained, if fresh tissues were *immersed* in the aforementioned lead solution for 15 min, sectioned, and treated subsequently with Na_2S. Other muscles (e.g. diaphragm, intercostal muscle, gastrocne-

[1] R. Couteaux, C. R. Soc. Biol. *127*, 571 (1938).
[2] R. Couteaux and D. Nachmansohn, Nature *142*, 1481 (1938).
[3] M. Crevier and L. F. Bélanger, Science *122*, 556 (1955).
[4] B. Csillik and Gy. Sávay, Acta morph. Acad. Sci. hung. *4*, 103 (1954).
[5] B. Csillik and Gy. Sávay, Acta neuroveget. *19*, 41 (1958).
[6] M. A. Gerebtzoff, Acta anat. *19*, 366 (1953).
[7] S. J. Holt, Proc. R. Soc. [B] *142*, 160 (1954).
[8] G. B. Koelle and F. S. Friedenwald, Proc. Soc. exp. Biol. Med., N.Y. *70*, 617 (1949).
[9] H. S. Koshtoyantz, 19th Internat. physiol. Congr. Montreal (1953).
[10] H. R. Downes, *The Chemistry of Living Cells* (Longmans, London 1955), p. 187, 509.

mius) and vegetative ganglia were successfully investigated by means of this immersion technique.

Three photomicrographs illustrate our results. Figure 1 shows some subneural apparatus from the intercostal muscle of the rat. Note characteristic lining of the subneural apparatus and the slight background reaction of muscle fibres. Figure 2 (high power) demonstrates the double-contured arborisations of the apparatus; here and there also the palisade-like organisation of the 'organites' appear. Figure 3 illustrates three ganglionic cells from the ciliary ganglion of the hen. Szentágothai *et al.*[11] demonstrated that the goblet-like synaptical structures of these ganglionic cells exhibit cholinesterase activity. Our method reveals a very strong reaction in these goblets, while cell bodies react only faintly.

Discussion. The native condition of the tissues seems to be indispensable for a histological demonstration of lead-reactive synaptical substances. It was found that neither fixed tissues, nor fresh frozen sections exhibited any reaction with lead salts. Destructive action of fixation and/or freezing on fine cytochemical structures had been observed also in our previous investigations concerning the microscopical localisation of potassium in nerve fibres[12].

With regard to the formalin and urea content of the lead-solution employed, it should be noted that these substances are not essential for the reaction. Formalin, however, renders possible a better histological localisation, in so far as thinner sections may be obtained. On the other hand, urea liberates buried –SH groups[10] which seem to be necessary for a complete reaction. Employing a lead-solution without urea, the results are less satisfactory.

As demonstrated by the photomicrographs, morphological localisation of lead-reactive synaptical structures is identical with that of cholinesterase activity. The question may arise whether lead-reaction is not due to cholinesterase[13]. Although the lead nitrate solution employed contains no substrate to be hydrolysed by the enzyme, lead ions possibly combine with some part of the cholinesterase molecule, e.g. with the supposed –SH groups of this enzyme (Nachmansohn and Lederer[14]). There are some observations, however, which oppose such an explanation. Cholinesterase activity is not affected by freezing, while lead-reaction is abolished by it. On the other hand, a pre-incubation in eserine (10^{-3} *M*, for 10 min) inhibits cholinesterase activity, while lead-reaction is not influenced by it. An isotonic solution of cadmium chloride, applied prior to the lead-reaction, abolishes it, while the cholinesterase activity remains unchanged. Finally, cholinesterase activity of motor end-plates may be demonstrated histochemically by means of indoxylacetate or thiolacetic acid in sections previously treated with a lead solution. It does not seem plausible, therefore, that lead reaction should be due to cholinesterase, but rather to another compound having the same histochemical localisation. Further investigations on this subject are in progress.

Gy. Sávay and B. Csillik

Department of Anatomy and Histology, Medical University, Szeged (Hungary), January 10, 1959.

[11] J. Szentágothai, Á. Donhoffer, and K. Rajkovits, Acta histochem. *1*, 272 (1955).

[12] B. Csillik and Gy. Sávay, Acta morph. Acad. Sci. hung. *8*, 3 (1958).

[13] Gy. Sávay and B. Csillik, Nature *181*, 1137 (1958).

[14] D. Nachmansohn and E. Lederer, C. R. Soc. Biol., Paris *130*, 321 (1939).

Zusammenfassung

Durch vitale bzw. supravitale Behandlung mit $Pb(NO_3)_2$ werden die subneuralen Apparate der quergestreiften Muskeln sowie die synaptischen Strukturen der vegetativen Ganglien dargestellt. Die Frage wird diskutiert, ob das bleireaktive Material mit den Sulfhydrylsubstanzen bzw. mit der Cholinesterase identisch sei.

Veränderungen der elektrischen Hirntätigkeit bei Katzen nach Röntgentotalbestrahlung [1]

Signifikante EEG-Veränderungen wurden bisher erst nach massiven Strahlendosen (4000–6000 r) gefunden[2–4], die ohnehin histologische Dauerschäden verursachen. Bei schwächeren Dosen konnten jedoch keine sicheren EEG-Veränderungen beobachtet werden[2]. Demgegenüber wird berichtet, dass die Strahlenempfindlichkeit bedingter Reflexvorgänge – insbesondere in komplexeren Testsituationen – auch bei schwachen Strahlendosen nachweisbar sei[5]. Es wurde deshalb untersucht, ob Veränderungen der elektrischen Hirntätigkeit nach Bestrahlung mit schwächeren Dosen erkennbar sind, wenn empfindlichere elektrophysiologische Messmethoden angewandt werden.

Methodik. Bei 5 frei beweglichen Katzen wurde die elektrische Hirntätigkeit mittels konzentrischer, stereotaktisch implantierter, chronischer Elektroden kortikal (sensomotorische und parietale Rinde) und subkortikal abgeleitet (mesenceph. subst. reticularis; thalamus: nucl. ventr. ant. (VA), centr. med. (CM) und centr. lat. (CL); hippocampus und nucl. amygdalae). Zugleich wurden nachfolgende Reizantworten auf elektrische Reizung untersucht und deren Schwellen bestimmt: 1. «Arousal reaction»[6] nach Reizung (150 Imp./sec, 1 msec) der mesencephalen subst. reticularis; 2. «Recruiting response»[7] nach Reizung (7 Imp./sec, 1 msec) des diffusen thalamischen Projektionssystems (VA, CM, CL); 3. Rhinencephale elektrische Nachentladung nach Reizung (150 Imp./sec, 1 msec) des Hippocampus oder des Mandelkerns. Alle Messungen wurden in einem schalldichten Käfig vorgenommen, wobei das Verhalten der Tiere durch eine Glasscheibe beobachtet werden konnte. Die Ableitung der elektrischen Hirntätigkeit erfolgte mit einem 8-Kanal-Offner-Tintenschreiber.

Gereizt wurde mit einem Grass-Impulsgenerator. Jeder Bestrahlung ging eine Kontrollperiode von 4 bis 6 Wochen voraus. Die Bestrahlung erfolgte mit zwei Picker-Einheiten simultan von oben und unten. Fokusabstand: 60 cm; Spannung: 250 KV, 15 mA; Dosisleistung: 43 r/min; Filterung: 0,5 mm Cu parabolisch und 1 mm Al; HWS: 1,7 mm; Gesamtdosis: 400 r Ganzkörperbestrahlung. Die Messungen setzten unmittelbar nach der Bestrahlung ein und wurden am gleichen Tag über 9 Stunden und weiterhin täglich zweimal fortgesetzt.

[1] This article is based on work performed under Contract No. AT-(04-1)-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

[2] E. Eldred and W. V. Trowbridge, EEG Clin. Neurophysiol. *5*, 259 (1953).

[3] J. A. T. Ross, S. R. Leavitt, E. A. Holst, and C. D. Clemente, A.M.A. Arch. Neurol. and Psychiat. *71*, 238 (1954).

[4] J. C. Helgason und R. S. Snider, Rad. Res. *3*, 267 (1955).

[5] M. I. Nemenov, Vestn. Rentgenol. Radiol. *26*, 43 (1944).

[6] G. Moruzzi und H. W. Magoun, EEE Clin. Neurophysiol. *1*, 455 (1949).

[7] R. S. Morison und E. W. Dempsey, Amer. J. Physiol. *135*, 281 (1942).

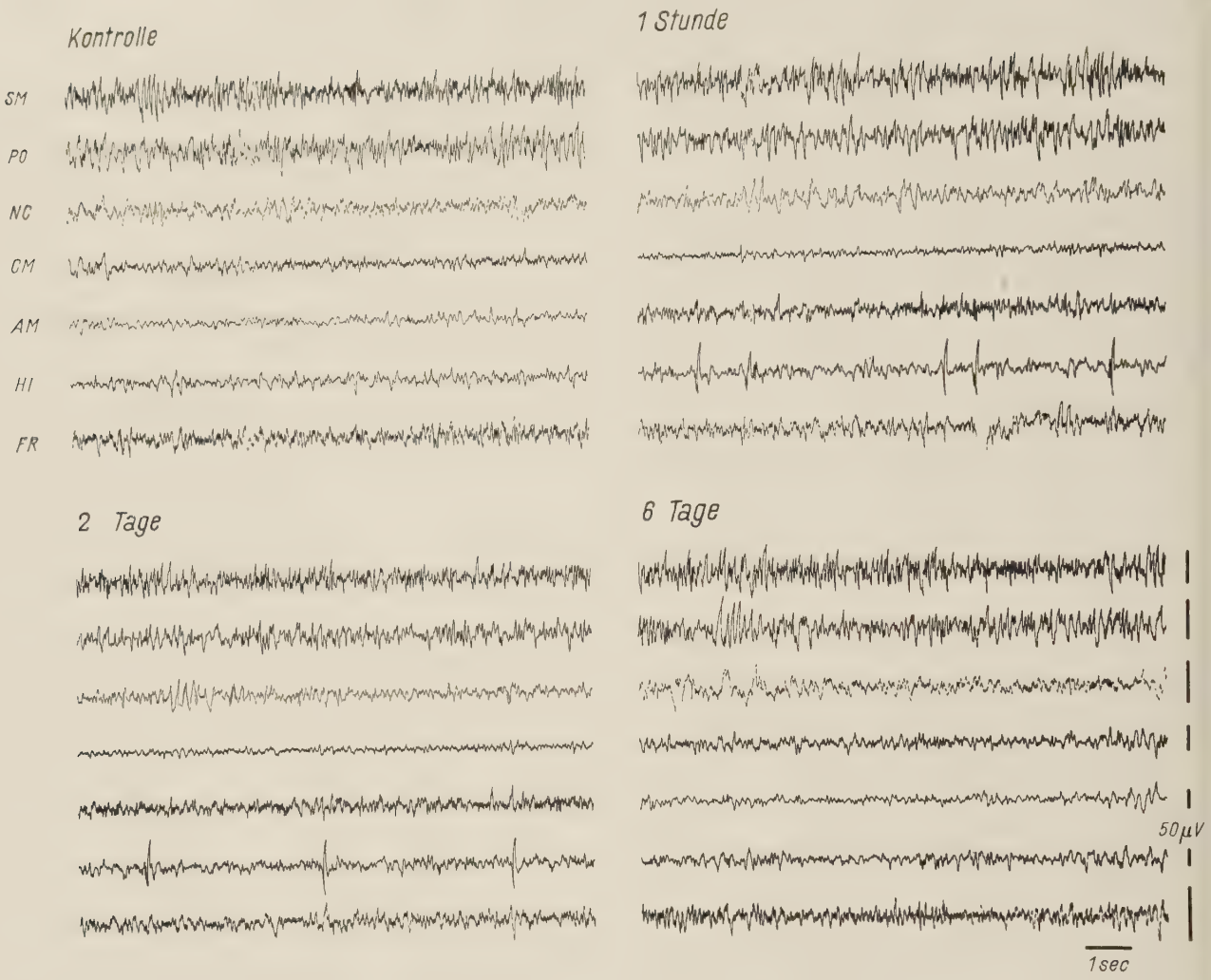


Abb. 1. Spontane EEG-Tätigkeit nach 400 r Ganzkörperbestrahlung. Lebhafte Spitzenentladungen im Hippocampus (HI) und im Mandelkern (AM) 1 h nach der Bestrahlung, die 2 Tage später im Hippocampus noch deutlich erkennbar sind. Normalisierung nach 6 Tagen. SM = sensomot. Rinde, PO = parietale Rinde, NC = Nucl. caudatus, CM = Nucl. centr. med. thalami, AM = Nucl. amygdalae, HI = dors. Hippocampus, FR = mesenceph. subst. reticularis

Ergebnisse

1. *Spontane, elektrische Hirntätigkeit:* Sofort nach der Bestrahlung zeigen die Tiere zunehmend frequente Spitzenentladungen im Hippocampus und im Nucleus amygdalae, die im Cortex nicht sichtbar sind (Abb. 1). Die Entladungsfrequenz dieser rhinencephalen Spitzen nimmt innerhalb der ersten zwei Stunden rasch zu, erreicht ein Maximum zwischen 3 und 7 h und nimmt nach 8 bis 9 h wieder rapid ab. Nach 3 Tagen ist dieser Effekt meistens völlig erloschen. Innerhalb der ersten 4 h ist ferner eine vorübergehende Zunahme der Wachrhythmen (low voltage fast waves) im EEG zu beobachten. Ansonsten zeigt das spontane EEG selbst nach Tagen und prämortal keinerlei signifikante, pathologische Veränderungen.

2. *Weckschwelle nach Reizung der Subst. reticularis:* Unmittelbar nach der Bestrahlung sinken die retikulären Weckschwellen rasch ab. Nach etwa 4 h kehren sie zur Ausgangslage zurück (Abb. 2). Während dieser ersten Stunden zeichnet sich das Tier durch gesteigerte Wachheit und durch das Vorherrschen von Wachrhythmen im spontanen EEG aus (s. o.). Während der nachfolgenden 2 bis 3 Tage entsprechen die Weckschwellen wie auch das Allgemeinverhalten der Tiere wieder etwa der Norm. Der Appetit ist noch ungestört und neurologische Ausfälle sind nicht erkennbar. Erst nach 4 bis 6 Tagen stellen sich Antriebsverarmung, Anorexie und ataktische Störungen ein. Alle Tiere gehen nach 5 bis 10 Tagen zugrunde. Nur selten wird jedoch ein Anstieg der Weckschwellen beobachtet.

3. *«Recruiting response» nach Thalamusreizung (VA, CM, CL):* Sicher verwertbare Veränderungen treten unmittelbar nach der Bestrahlung nicht auf. Allenfalls ist ein geringer Anstieg der Schwellenwerte am Ende des ersten Tages bemerkbar. Nach 2 bis 4 Tagen steigen jedoch alle Thalamusschwellen für Rekrutierung signifikant um 2–4 Volt an.

4. *Die Hippocampus-Nachentladung:* Signifikante Schwellenwertveränderungen haben sich bislang nicht nachweisen lassen.

Besprechung: Bei Ganzkörperbestrahlung mit relativ schwachen Röntgendosen treten eindeutige Veränderungen der elektrischen Hirntätigkeit vorwiegend subkortikal auf, die sich im spontanen kortikalen EEG nicht widerspiegeln. Wir unterscheiden: 1. Rückbildungsfähige, vorübergehende Frühveränderungen (rhinencephale Spitzenentladungen, Absinken der retikulären Weckschwelle), die dann auftreten, wenn das Allgemeinbefinden noch nicht gestört ist. Die Rückbildungsfähigkeit dieser Veränderungen lässt vermuten, dass es sich um funktionelle Vorgänge handelt, die möglicherweise mit vorübergehenden Veränderungen im Chemismus der betroffenen Hirnstrukturen verknüpft sind. Über die Natur dieser Prozesse lässt sich heute noch nichts Sicheres sagen. – 2. Spätveränderungen (Anstieg

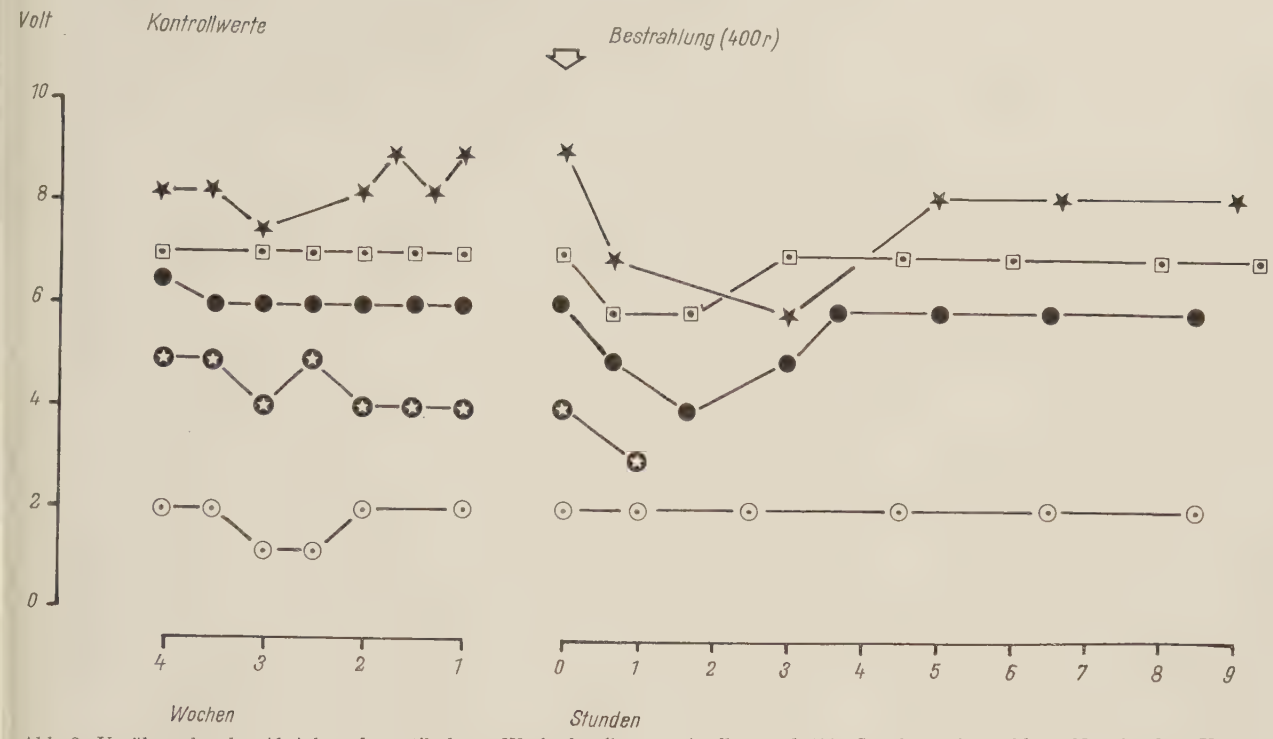


Abb. 2. Vorübergehendes Absinken der retikulären Weckschwellen unmittelbar nach 400 r Ganzkörperbestrahlung. Nur eine der 5 Katzen zeigt keinerlei Veränderung der Schwellenwerte. Man beachte jedoch die schon primär niedrigen Kontrollschwellenwerte (1 und 2 Volt) bei diesem Tier.

der Thalamusschwelle für Rekrutierung, gelegentlicher Anstieg der retikulären Weckschwelle), die zeitlich teilweise mit Störungen des Allgemeinbefindens, des Appetits, des Antriebs und der Koordination zusammenfallen und Folge einer organischen Gewebsschädigung sein könnten. Die diesbezüglichen, histologischen Ergebnisse sollen gesondert mitgeteilt werden. – Inwieweit die beschriebenen Veränderungen peripher ausgelöste oder direkt zentrale Vorgänge sind, werden weitere Untersuchungen klären. Zur Zeit sind Untersuchungen mit noch schwächeren Dosen im Gang.

H. Gangloff und T. J. Haley

Abteilung für Anatomie und Abteilung für nukleare Medizin der Universität Kalifornien, Los Angeles, 20. April 1959.

Summary

In cats with chronically implanted electrodes, hippocampus and amygdala consistently showed spike discharges immediately after relatively low doses of total body x-irradiation (400 r) and reticular arousal thresholds were transitorily decreased. Thalamic recruiting thresholds increased later, while hippocampal seizure thresholds remained practically unaltered. Experiments with lower doses are in progress.

Optically Aimed Ear Movements in the Rabbit

It is well known that rabbits have mobile auricles. Early investigators laid emphasis on the reflex-like character of the observed movements. Högyes[1] described the 'Preyer-reflex' for the rabbit, a stereotype ear movement caused by tones of high frequency. Horsley[2] described such a movement, evoked by mechanical stimulation (pressure on infra-orbital region). Several authors (Mangold[3]) pointed to another type of ear movement, a directioning of the auricle to the sound-source. Such directed ear movements are encountered frequently when observing rabbits. They may even appear when an object that probably has no acoustical value for the rabbit, is passing through its visual field.

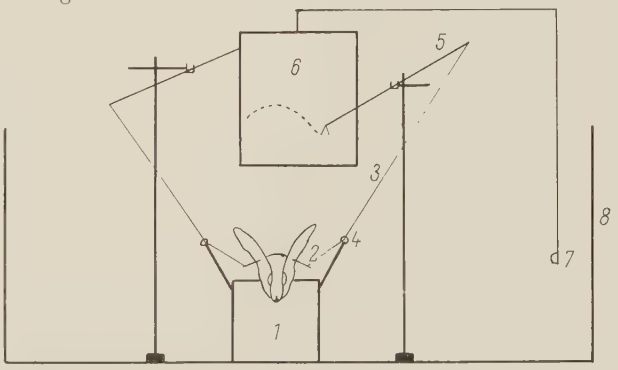


Fig. 1. – Experimental Set

1 fixed rabbit; *2* piece of straw attached to auricle; *3* thread that connects straw with recorder; *4* ring through which thread is conducted; *5* recorder; *6* kymograph-drum; *7* light-source; *8* enclosing black wall

This observation led to a series of experiments in which the possibility of an optical causation of the ear movements was examined. In a darkened room, a 2 V, 0·05 A light-source circled at constant speed around a fixed rabbit[4]. Ear movements were recorded on a kymograph

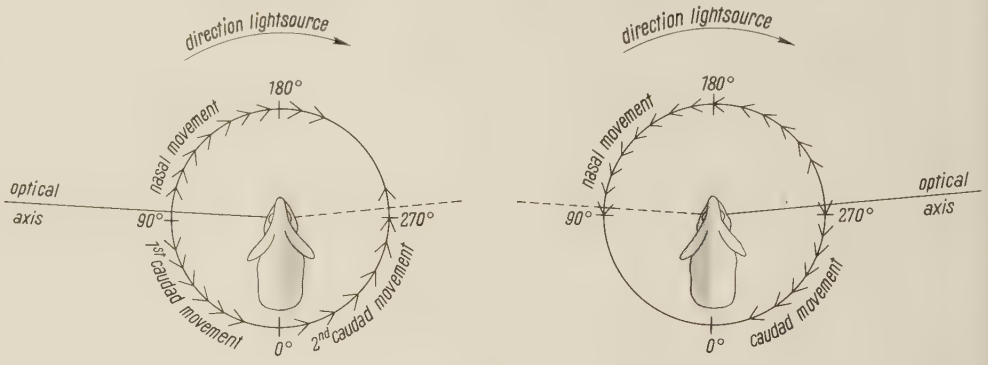
[1] A. Högyes, Zbl Nervenheilk. *9*, 268 (1886).

[2] V. Horsley, Brain *28*, 65 (1905).

[3] E. Mangold, *Wintersteins Handbuch der vergleichenden Physiologie* (Jena 1913).

[4] Three rabbits were each individually subjected to as many experiments.

Fig. 2. – Direction Ear Movement in Relation to Angle passed through by Light-Source



	Auricle at the side of the rabbit that is first passed by the light-source						Auricle at the side of the rabbit that is next passed by the light-source					
	1st caudad movement		nasal movement		2nd caudad movement		1st temporal movement		nasal movement		2nd caudad movement	
Frequency	7/12		12/12		5/12		0		12/12		9/12	
	start	end	start	end	start	end	start	end	start	end	start	end
Mean Value	20°36′	74°	108°	204°12′	262°48′	345°36′			90°	240°	268°48′	340°48′
Standard Deviation	18°54′	38°	35°	40°36′	35°54′	18°18′			39°54′	27°12′	6°48′	20°18′

In half of the experiments, the direction of the circular movement of the light-source was clockwise, in the other half it was anti-clockwise. Each movement started, and came after one turn to an end, behind the rabbit. The results are converted in such a way that in the Figure they all correspond to one direction of the light-source. Arbitrarily, the clockwise direction is chosen. In each half of the Figure, movements of one ear are shown. The movements of the ear at the side first passed by the lamp (in the Figure the left side of the rabbit) are shown in the left half of the Figure. The movements of the ear at the side that is next passed by the lamp (in the Figure the right side of the rabbit) are shown in the right half of the Figure. The track of the light-source is indicated by the circular lines; the direction of the ear movement is indicated by the arrows drawn in these lines.

drum. For this purpose, a piece of straw was fixed at the base of each auricle. The straws were connected by thread with recorders (Fig. 1). If the circling lamp was not burning, no movements, or at most slight random movements were recorded. The same applies to the recordings made when the circling lamp was burning but the eyes of the rabbit were blindfolded. However, when the lamp was burning and when the eyes were uncovered, clear ear movements were recorded. These results justify the conclusion that optical stimuli can cause ear movements.

In a second series of experiments, the direction of the ear movement (nasal or caudal) was examined in relation to the angle passed through by the lamp. To this end 12 experiments were performed[4]. In all experiments the speed of the lamp was 7° 30′/s[5]. In half of the experiments the lamp moved in one direction, in the other half it moved in the opposite direction. As was expected, the recordings of the former group of experiments were approximately the image of those of the latter group. In Figure 2 the results are converted in such a way that they all correspond to one direction of the lamp. This Figure shows that if the lamp moved in the nasal part of the visual field of one of the eyes, the homo-lateral auricle moved in the nasal direction. It never moved in the opposite way. If the lamp moved in the caudal part of this visual field, the auricle, if reacting, moved in the caudal direction. Also in this case, it never moved in the opposite way. The conclusion seems justified that the direction of the ear movement is primarily determined by the area of the retina that is stimulated. The direction of the image movement on the retina seems to have no influence on the direction of the ear movement. As becomes evident from Figure 2, the movement of an auricle is for the greater part directed by the homo-lateral eye, although the contra-lateral eye also has an influence on its direction. These results justify the conclusion that the recorded ear movements are optically directed.

As is known, the rabbit's retina has no area centralis. Probably the most important function of the eye is detection of moving objects. The function of the described ear movement will be a rough aiming of the auricles at moving objects to get acoustic information, if any. Probably the finer adjustments will be acoustically directed.

J. F. W. Nuboer

Laboratorium voor vergelijkende Physiologie der Rijks-Universiteit, Utrecht, June 4, 1959.

Zusammenfassung

Durch Bewegungssehen ausgelöste Ohrmuschelbewegungen beim Kaninchen werden registriert. Die Ohrmuscheln werden dem Objekt zugewendet. Umgekehrt wie beim Menschen, wo akustische Reize eine gerichtete Blickwendung auszulösen vermögen, erfolgt hier auf visuellen Reiz hin eine gerichtete Ohrmuschelwendung.

[5] In preliminary experiments the following speeds were used: 50′/s, 3° 45′/s, 7° 30′/s, 60°/s, and 120°/s. Of these speeds that of 7° 30′/s proved to give best results.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Atom Transfer Mechanisms in Oxidation Processes*

By R. Stewart

Abstract

The emphasis placed on electron transfer in connection with oxidation reactions has often resulted in these processes being considered as a group apart from the main class of chemical reactions. Although some oxidations, for example certain exchange reactions between ions in solution, can be reasonably described as electron transfer processes, there are very many other oxidations, whose mechanisms are much better described in the familiar terms of modern organic chemistry.

The use of isotopes as tracers has shown, that many oxidations proceed with transfer of atoms or groups from oxidant to reductant and *vice versa*; the use of the kinetic isotope effect has shown that such transfer is seldom an incidental process, but is almost always a part of the slow step of the reaction. In this paper, oxidations involving the transfer of such species as oxygen atoms, hydride ions, hydrogen atoms, chlorine atoms, and hydroxyl radicals are discussed in terms of mechanism. An attempt is made to show that a graded series of mechanisms is possible ranging from what appear to be pure electron transfer processes at one end to certain atom transfer processes at the other. The latter group belong, in fact, in the familiar realm of ordinary chemical raactions, in which strong bonds are being broken and formed in the activated complex.

The concept of oxidation-reduction as an electron transfer process has been strongly emphasized for some time with the result that oxidation processes have come to be considered as a group apart from the main class of chemical reactions. An enormous amount of information has accumulated in the last two or three decades regarding the mechanisms of non-oxidative processes particularly in organic chemistry but only recently have oxidation reactions come under close scrutiny. The use of isotopes has shown that many oxidations of both organic and inorganic substrates proceed with transfer of atoms or groups from oxidant to reductant and *vice versa*. One way to distinguish oxidations from all other chemical reactions except electron excitation processes is to visualize them as occurring by purely electronic transitions not involving bond making or bond breaking except, in some cases, as mere adjuncts to the electron transfer. Just as the use of isotopes has demonstrated that transfer of atoms often occurs between oxidant and reductant, so the use of the kinetic isotope effect has demonstrated that the bond breaking which often accompanies an oxidation is seldom an incidental process to the electron transfer but is rather almost always a part of the slow step of the reaction.

A recent suggestion that 'the true mechanism of organic oxidation may well be identical with the well-recognized fundamental *definition* of oxidation, i.e., *a loss of electrons*'[1] seems unfortunate. It appears in fact that many oxidations of both organic and inorganic substrates have mechanisms which are readily explicable in the terms used to describe the well known reactions of organic chemistry[2]. For example Anbar and Taube using O^{18} have shown that the oxidation of nitrite by hypochlorite in aqueous solution takes place with substantially complete transfer of oxygen to nitrite[3]. The mechanism appears to be a displacement on the oxygen of the hypochlorite, the nitrite ion displacing chloride in the manner of an SN_2 reaction,

$$NO_2^- + OCl^- \longrightarrow [O_2N\text{---}O\text{---}Cl]^= \longrightarrow NO_3^- + Cl^-$$

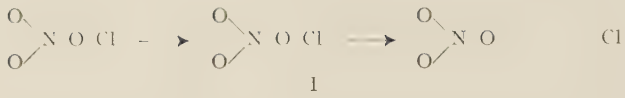
The orbitals which are used to bind the atoms in the activated complex are not known with any certainty but it appears reasonable that the use of *p* orbitals on oxygen would provide a convenient path for the reaction.

Certain reactions, of course, appear to be well described as electron transfers, in particular the exchange reactions in solution between ions of the same metal but of different valence. There is a possible difficulty in the way of an arbitrary distinction between electron and atom transfer mechanisms. The exchange which occurs between $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$ can be followed radiochemically and is rapid as it is for the similar systems MnO_4^{-}–MnO_4^{-2}, $IrCl_6^{-3}$–$IrCl_6^{-2}$, and $W(CN)_6^{-3}$–$W(CN)_6^{-2}$, $Os(dipy)_3^{+2}$–$Os(dipy)_3^{+3}$ [4–6]. Electron transfer is the obvious path for these reactions yet even here it is easy to show that some mass transfer probably accompanies the electron transfer. Taking the ferricyanide-ferrocyanide case as an example it is clear that in aqueous solution the more highly charged ferrocyanide ion will be more highly solvated than will the ferricyanide ion. When the electron transfer occurs, however, the positions are reversed and undoubtedly some water molecules which had been solvating the ferrocyanide ion before reaction and solvating both ions in the activated complex will change their allegiance to the newly formed ferricyanide ion when the activated complex splits. Such a transfer of molecules from the second coordination sphere of one metal ion to the second coordination sphere of the other may seem a trivial affair considering that exchange of solvating molecules with bulk solvent occurs readily and considering that the forces involved must be small for ions of such size. Yet one can imagine a graded series of reactions in which the coordinated groups which are transferred vary from being loosely bound to the central atoms to being tightly bound. The case of oxygen atom transfer from hypochlorite to nitrite is in the latter category.

When we come as far along the graded series as the nitrite-hypochlorite reaction we find ourselves in the familiar realm of ordinary chemical reactions in which strong bonds are being broken and formed in the activated complex. Thus the nitrite ion as it approaches the hypochlorite ion begins to alter its bond lengths and bond angles so that in the activated complex its geometry resembles more that of the trigonal nitrate ion. The changes in geometry are quite small in this case which doubtless is a factor favouring the reaction.

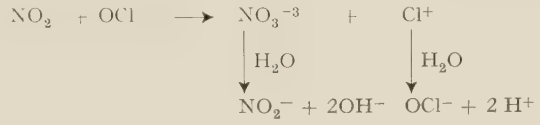
* Contribution from Department of Chemistry, University of British Columbia, Vancouver. — Presented at the Organic Chemistry Symposium of the Chemical Institute of Canada, Ottawa, Dec. 8–9, 1958.

[1] L. S. Levitt, J. org. Chem. *20*, 1297 (1955).
[2] C. K. Ingold, Chem. Rev. *15*, 266 (1934).
[3] M. Anbar and H. Taube, J. Amer. chem. Soc. *80*, 1073 (1958).
[4] A. C. Wahl and C. P. Deck, J. Amer. chem. Soc. *76*, 4054 (1954).
[5] J. C. Shepherd and A. C. Wahl, J. Amer. chem. Soc. *79*, 1020 (1957).
[6] E. Eichler and A. C. Wahl, J. Amer. chem. Soc. *80*, 4145 (1958).



I

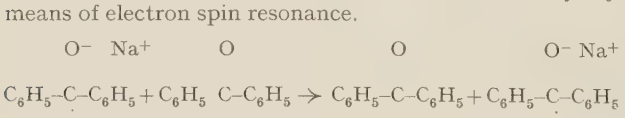
Let us consider the following hypothetical reaction which is merely exchange of oxygen between nitrite and hypochlorite i.e., oxide ion transfer without attendant oxidation or reduction.



The arrangement of atoms in the activated complex should be much the same as in I but a different electron distribution is required, an impossible situation since the electrons will automatically take up positions of minimum energy. In all chemical reactions the atoms in the reacting molecules take up different relative positions to one another when they form the activated complex and oxidation occurs when the electron distribution of minimum energy is such that the products formed on scission of the complex are in different oxidation states than they were before reaction.

Marcus[7] has pointed out that unlike most chemical reactions the so-called electron transfer processes, typified by the $Fe(CN)_6^{-4}$–$Fe(CN)_6^{-3}$ exchange, require only a weak electronic interaction of the reactants to couple them electronically and so permit electron transfer to occur. The formal similarity between photochemical excitation on the one hand and these electron transfer processes on the other has led to the application of the Franck-Condon principle to the study of these reactions, in particular the restrictions placed by this principle, which states that atomic motions are slow compared to the speed of an electron jump, on electron transfer between solvated ions[8]. If the ferrous-ferric ion exchange in aqueous solution occurs by electron transfer it has been shown that considerable reorganization of the solvation spheres is required prior to the electron transfer to keep the overall free energy change zero. This is because the two cations have different charges and hence have their solvation spheres at different distances. If a direct electron transfer occurred between normally solvated Fe^{+2} and Fe^{+3} the products would be formed in excited states and hence the overall free energy change would not be zero as required. This and other aspects of inorganic oxidation are discussed by Basolo and Pearson[9].

Much attention has recently been devoted to these reactions, which can be fairly labelled electron transfer processes, and considerable success has been achieved in their quantitative treatment[10,11]. It should be pointed out however that recent work by Adam and Weissman[12] indicates that atom transfer is a better way to describe at least one case of exchange in solution. They studied the exchange between benzophenone and its sodium ketyl by means of electron spin resonance.



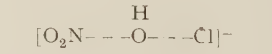
They showed that the spin resonance spectrum of the ketyl which is split by the nuclear spin of the nearby Na^{23} nucleus is greatly altered in the presence of excess benzophenone. It is altered in such a way that the exchange which occurs must result from each electron which is transferred from ketyl to ketone being accompanied by a sodium nucleus, in other words, sodium atom transfer from ketyl to ketone.

The purpose of this article then is to consider some oxidations which are not adequately described as electron transfer processes and to emphasize the similarity which exists between the mechanisms of these reactions and those of ordinary non-oxidative chemical processes.

1- and 2-Equivalent Oxidations. As early as 1929 Kirk and Browne distinguished between reagents which could oxidize in 1- and 2-equivalent steps by a study of the products formed when hydrazine is oxidized[13].

Kirk and Browne used the terms mono de-electronator and di-de-electronator and others have called them 1- and 2-electron transfer reagents but as Higginson and Marshall[14] point out these terms imply that the reaction necessarily occurs via electron transfer; they therefore prefer the designations 1- and 2-equivalent oxidants or reductants. Some reagents can function as either 1- or 2-equivalent reagents and subsequent work supports Kirk and Browne's concept[15,16]. Westheimer[17] has made the following distinction between 1- and 2-equivalent reactions. If a molecule with an even number of electrons is oxidized or reduced to another molecule with an even number of electrons the process is considered to be a 2-equivalent oxidation unless a species is produced which contains an odd number of electrons and has a half life of $> 10^{-11}$ s. The simultaneous transfer of two electrons is considered improbable on quantum mechanical grounds but since atomic motion is slow compared to electronic motion any oxidation which proceeds by way of atom transfer can also involve a 2-electron transfer without the latter being considered 'simultaneous'. The question then arises must all 2-equivalent reactions proceed with atom transfer. It appears that most if not all of them do. One equivalent oxidation reactions on the other hand might proceed with or without atom transfer.

Oxygen Atom Transfer to Reductant. Taube's work with O^{18} has demonstrated that oxygen transfer is an important mode of oxidation in solution. The nitrite-hypochlorite reaction referred to earlier is catalyzed by acid and it seems reasonable to locate the proton in the activated complex on the oxygen atom being transferred[3].



The resulting decrease in coulombic repulsion between the anions is doubtless an important factor but there are several oxidation reactions between anions which proceed with oxygen transfer and are inhibited by acid. The oxidation of cyanide ion by permanganate ion in basic solution results in transfer of oxygen from the permanganate to cyanide. Below pH 8 where the cyanide exists almost completely as HCN the reaction is very slow[18].

[7] R. A. Marcus, Trans. N.Y. Acad. Sci. *19*, 432 (1957).

[8] W. F. Libby, J. phys. Chem. *56*, 863 (1952).

[9] F. Basolo and R. G. Pearson, *Mechanisms of Inorganic Reactions* (John Wiley & Son, New York 1958), p. 303.

[10] B. J. Zwolinski, R. J. Marcus, and H. Eyring, Chem. Rev. *55*, 157 (1955).

[11] R. A. Marcus, J. chem. Phys. *26*, 872 (1957).

[12] F. C. Adam and S. Weissman, J. Amer. chem. Soc. *80*, 1518 (1958).

[13] R. E. Kirk and A. W. Browne, J. Amer. chem. Soc. *50*, 337 (1928).

[14] W. C. E. Higginson and J. W. Marshall, J. chem. Soc. *1957*, 447.

[15] W. C. E. Higginson, D. Sutton, and P. Wright, J. chem. Soc. *1953*, 1380.

[16] J. Halpern, Can. J. Chem. *37*, 148 (1959).

[17] F. H. Westheimer, *The Mechanisms of Enzyme Action* (Ed. W. D. McElroy and B. Glass, John Hopkins Univ. Press) p. 321.

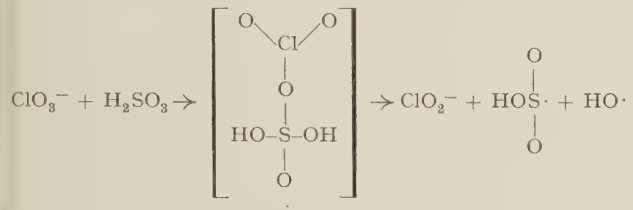
[18] R. Stewart and R. Van Der Linden, unpublished results.

Similarly formate ion is oxidized by permanganate ion faster than is formic acid. Some oxygen transfer also occurs in this reaction[19].

In basic solution hypochlorite oxidizes sulfite with oxygen transfer being the principal reaction path. In acid solution however displacement on the chlorine atom of the hypochlorite appears to be favoured[20].

$$SO_3^{-2} + ClO^- \xrightarrow{H^+} [O_3S\cdots Cl - O\cdot H]^{-2} \rightarrow SO_3Cl^- + OH\cdot$$
$$SO_3Cl^- + OH^- \longrightarrow SO_4^{-2} + H^+ + Cl^-$$

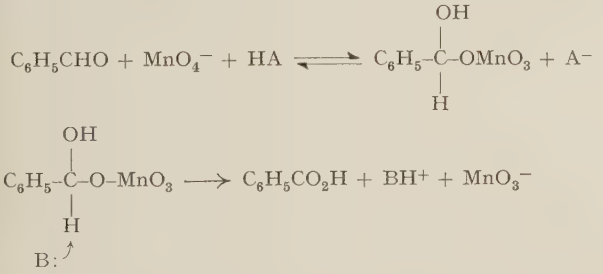
Taube *et al.* have also demonstrated transfer of oxygen to sulfite from BrO_3^-, ClO_2^-, ClO_3^-, and H_2O_2. In acid solution the latter reagent transfers both its oxygen atoms to the substrate[21]. It has been suggested that the sulfite-chlorate system for one involves more than a simple oxygen transfer since in the pH range of 1–3·5 it can initiate polymerization[22,23] a diagnostic test for the presence of free radical intermediates. To accommodate the known oxygen transfer with the presence of radical intermediates the following mechanism has been proposed[24].



$$HSO_3\cdot + HO\cdot \longrightarrow 2H^+ + SO_4^{-2}$$
$$HSO_3\cdot \text{ or } HO\cdot + \text{monomer} \longrightarrow \text{polymer.}$$

The scission of the above activated complex to give ClO_2^-, $HSO_3\cdot$ and $HO\cdot$ rather than ClO_2^-, $2H^+$ and SO_4^{-2} seems surprising. The radicals which initiate polymerization may be formed in small amount by a competing side reaction rather than by the principal, oxygen transfer, route.

Turning to an organic substrate, benzaldehyde, which is oxidized by permanganate to benzoic acid, we find that a somewhat different mechanism is required. The mechanism must be in accord with the fact that O^{18} from MnO_4^{18-} is found in the benzoic acid formed in the reaction and also with the fact that a large isotope effect (7:1) is observed when C_6H_5CDO is oxidized by permanganate. The following mechanism which is also in accord with the reaction kinetics has been suggested[25].



$$C_6H_5CHO + MnO_4^- + HA \rightleftharpoons C_6H_5{-}\underset{\displaystyle H}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}{-}OMnO_3 + A^-$$

$$C_6H_5{-}\underset{\displaystyle H}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}{-}O{-}MnO_3 \longrightarrow C_6H_5CO_2H + BH^+ + MnO_3^-$$

B: ↷

Oxygen atom transfer occurs in this process but the rate controlling step of the reaction is a series of electron pair shifts, to use the common terminology of organic chemistry, which is initiated by proton removal by the base B:. The kinetic isotope effect shows that the latter operation is a part of the rate controlling step.

The above mechanism is similar to that elucidated by the important work of Westheimer on the chromate oxidation of isopropyl alcohol, the mechanism of which is[26]:

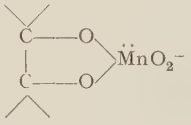
$$(CH_3)_2CHOH + 2H^+ + CrO_4^= \rightleftharpoons (CH_3)_2\underset{\displaystyle H}{\overset{|}{C}}OCrO_3H_2^+$$

$$(CH_3)_2\underset{\displaystyle H}{\overset{|}{C}}OCrO_3H_2^+ \longrightarrow (CH_3)_2CO + BH^+ + H_2CrO_3$$

B: ↷

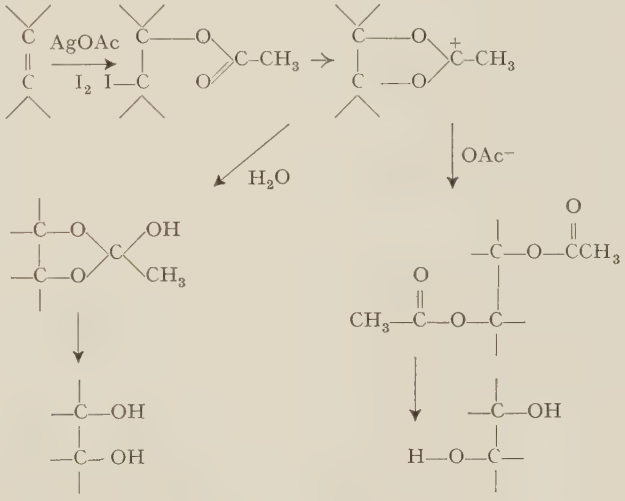
The proof of formation of the intermediate chromate ester, the existence of a large kinetic isotope effect, the existence of base catalysis and the form of the reaction kinetics all give strong support to this mechanism[27–29]. It is clear however that here any introduction of oxygen is merely a consequence of the mode of formation of the chromate ester. The rate controlling step of the oxidation involves proton removal and a shift of electrons. (See however Rocek[30].)

Permanganate ion behaves as an oxygen donor when it oxidizes olefins to glycols. It was early postulated[31,32] that a cyclic ester is the intermediate, and recent work has shown that the



oxygen in the glycol comes from the permanganate, not from the solvent[33].

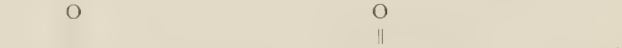
The mechanism of a similar reaction, the Prevost oxidation of olefins to glycols using silver acetate and iodine has been confirmed recently by the use of O^{18} as



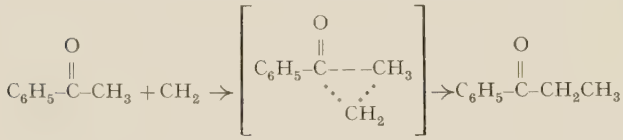
[19] K. B. Wiberg and R. Stewart, J. Amer. chem. Soc. *78*, 1214 (1956).
[20] J. Halperin and H. Taube, J. Amer. chem. Soc. *74*, 375 (1952).
[21] J. Halperin and H. Taube, J. Amer. chem. Soc. *74*, 380 (1952).
[22] A. Cresswell, U. S. Patent 2,751,374 (1956).
[23] A. Hill, U. S. Patent 2,673,192 (1954).
[24] E. H. Gleason, G. Mino, and W. M. Thomas, J. phys. Chem. *61*, 444 (1957).
[25] K. B. Wiberg and R. Stewart, J. Amer. chem. Soc. *77*, 1788 (1955).
[26] F. H. Westheimer, Chem. Rev. *45*, 419 (1950).
[27] F. H. Westheimer and N. Nicolaides, J. Amer. chem. Soc. *71*, 25 (1949).
[28] F. Holloway, M. Cohen, and F. H. Westheimer, J. Amer. chem. Soc. *73*, 65 (1951).
[29] L. Kaplan, J. Amer. chem. Soc. *77*, 5469 (1955).
[30] J. Rocek and J. Krupicka, Chem. & Ind. *1957*, 1668.
[31] G. Wagner, J. Russ. phys. chem. Soc. *27*, 219 (1895).
[32] J. Boeseken, Rec. Trav. chim. Pays-Bas *40*, 553 (1921).
[33] K. B. Wiberg and K. A. Saegebarth, J. Amer. chem. Soc. *79*, 2822 (1957).

tracer. The use of 'wet' or 'dry' conditions determines the stereochemistry of, and the source of oxygen in, the product[34].

The perbenzoic acid oxidation of benzophenone to phenyl benzoate has been shown to take place with the carbonyl oxygen in the ketone becoming the carbonyl oxygen in the ester[35].

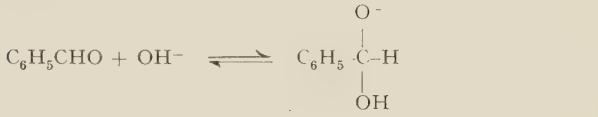


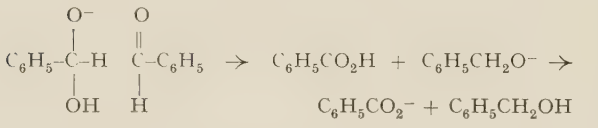
This insertion of an oxygen atom between two bonded carbon atoms is similar to the behaviour of the carbene, CH_2, produced by the irradiation of diazomethane[36].



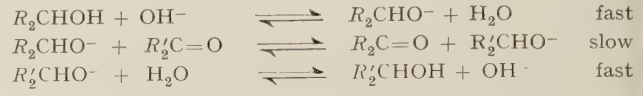
It is of interest that O and CH_2 are isoelectronic.

Hydride Transfer to Oxidant. A hydride reaction is the transfer in a single step of a proton and two electrons from reducing agent to oxidizing agent. Reactions believed to take place by this mechanism are very common in organic chemistry. They include the Cannizzaro reaction[37], the Meerwein-Pondorff reduction and its analog the Oppenauer oxidation[38], the Sommelet reaction[39], the Leuckart reaction[40], the base catalyzed carbinol-carbonyl equilibrium[41], the reduction by Grignard reagents of hindered ketones[42], and others[43–45]. The Cannizzaro reaction is often written as proceeding through a hydride transfer to benzaldehyde from the conjugate base of benzaldehyde hydrate[37,46]. The low deuterium isotope effect, $k_H k_D = 1{\cdot}8$[47], found for



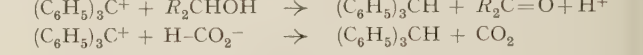


this reaction when C_6H_5CDO is used is surprising but may still be in accord with this mechanism (see later section). Doering and Aschner showed that the base catalyzed carbinol-carbonyl equilibrium is a 2-equivalent reaction by demonstrating the indifference of the reacting centers to the presence of a radical, Bindschedler's Green. Either a 1-electron transfer or a hydrogen atom transfer should involve this reactive species[41].



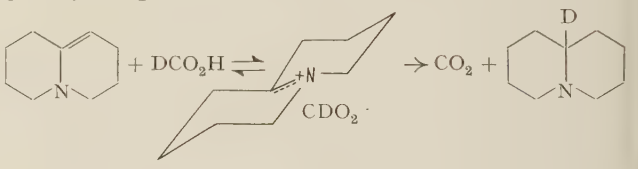
An interesting intramolecular reaction of this type has recently been reported by Dvornik and Edwards[48].

Although a hydride transfer is a most reasonable mechanism to write for the organic reactions listed earlier, there is some difficulty in obtaining proof that this is indeed the reaction path. In all cases equilibria prior to the rate controlling step hinder a thorough study of the reaction. Recently kinetic and isotopic evidence in support of the hydride transfer mechanism has been obtained by a study of the reduction of the triphenylmethyl cation by alcohols in strongly acid solution[49] and by formate ion[50] in formic acid.



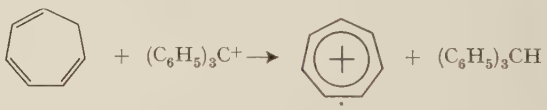
The use of R_2CDOH and DCO_2H resulted in the production of $(C_6H_5)_3CD$ thus fixing the position of the hydrogen transferred in the reduction.

Leonard and Sauers[51] have shown that the formic acid reduction of enamines proceeds by a similar mechanism and it seems likely that the Leuckart reaction also goes by this path.



The reduction of diazonium salts by alcohols appears to take place by both a hydride transfer mechanism analogous to the carbonium ion reductions above and by a free radical chain process[52].

Hydride abstraction by carbonium ions has been used to prepare tropylium ions from cycloheptatriene[53].



An interesting oxidation which bears a marked formal resemblance to hydride transfer is the reaction of hydrazoic acid with nitrous acid in acid solution.

Tracer work with N^{15} has shown that the reaction path is probably the following[54].

[34] K. B. Wiberg and K. A. Saegebarth, J. Amer. chem. Soc. *79*, 6256 (1957).

[35] W. von E. Doering and E. Dorfman, J. Amer. chem. Soc. *75*, 5595 (1953).

[36] E. Mosettig and L. Jovanovic, Mh. Chem. *53*, 427 (1929).

[37] L. P. Hammett, *Physical Organic Chemistry* (McGraw Hill Book Co., N.Y. 1940), p. 350.

[38] R. B. Woodward, N. L. Wendler and F. J. Brutschy, J. Amer. chem. Soc. *67*, 1425 (1945).

[39] S. J. Angyal and R. C. Rassack, J. chem. Soc. *1949* (2700).

[40] M. L. Moore in *Organic Reactions*, Vol. V (R. Adams, Ed., John Wiley and Sons, New York 1949), p. 301.

[41] W. von E. Doering and T. C. Aschner, J. Amer. chem. Soc. *75*, 393 (1953).

[42] G. E. Dunn and J. Warkentin, Canad. J. Chem. *34*, 75 (1956).

[43] R. Stewart, J. Amer. chem. Soc. *79*, 3057 (1957).

[44] M. Avranoff and Y. Springzak, J. Amer. chem. Soc. *80*, 493 (1958).

[45] P. D. Bartlett, F. E. Condon, and A. Schneider, J. Amer. chem. Soc. *66*, 1531 (1954).

[46] T. A. Geissman in *Organic Reactions*, Vol. II (R. Adams, Ed. John Wiley and Sons, New York 1944), p. 96.

[47] K. B. Wiberg, J. Amer. chem. Soc. *76*, 5371 (1954).

[48] D. Dvornik and O. E. Edwards, Proc. chem. Soc. *1958*, 280.

[49] P. D. Bartlett and J. D. McCollum, J. Amer. chem. Soc. *78*, 1441 (1956).

[50] R. Stewart, Canad. J. Chem. *35*, 766 (1957).

[51] N. J. Leonard and R. R. Sauers, J. Amer. chem. Soc. *79*, 6210 (1957).

[52] D. F. De Tar and T. Kosuge, J. Amer. chem. Soc. *80*, 6072 (1958).

[53] H. J. Dauben, F. A. Gadecki, K. M. Harmon, and D. L. Pearson, J. Amer. chem. Soc. *79*, 4557 (1957).

[54] K. Clusius and E. Eppenberger, Helv. chim. Acta *38*, 1843 (1955).

$$N_3^- + NO^+ \longrightarrow [NNNNO] \longrightarrow N_2 + N_2O$$

This reaction is effectively a nitride transfer, N^-, from azide ion to the oxidizing agent, NO^+. Like the hydride ion the nitride ion despite its being isoelectronic with atomic oxygen causes a 2-equivalent reduction. Transfer of nitrogen of any charge other than -3 will result in an oxidation or reduction. This applies also to transfer of oxygen, halogen, and hydrogen of charge other than -2, -1, and $+1$ respectively and results from our quite artificial concept of oxidation-reduction.

Molecular hydrogen in solution can bring about reduction by a homogeneous process. Cupric ion is not only reduced itself by hydrogen in aqueous solution but catalyzes the reduction of such substrates as $Cr_2O_7^{-2}$, IO_3^- and Ce^{+4}. The mechanism appears to be the following, the first step being a hydride transfer[55].

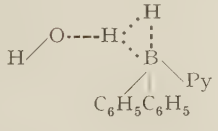
$$Cu^{+2} + H_2 \rightleftharpoons CuH^+ + H^+$$
$$CuH^+ + Cu^{+2} \longrightarrow 2\,Cu^+ + H^+$$
$$2\,Cu^+ + \text{substrate} \longrightarrow \text{products} + 2\,Cu^{+2}$$

Although the evidence for the formation of CuH^+ is indirect, that is, the ion CuH^+ has not been isolated, the above mechanism fits the kinetic data very nicely.

It has recently been pointed out that small deuterium kinetic isotope effects seem to be the rule for hydride transfer reactions[56] (see however Ref. [43]). The hydrolysis of pyridine diphenylborane is assumed to go by the following mechanism:

$$(C_6H_5)_2\bar{B}\begin{matrix}H\\ \diagup\\ \diagdown\\ Py^+\end{matrix} + H_2O \longrightarrow (C_6H_5)_2BPy^+ + H_2 + OH^-$$

Hawthorne and Lewis explain the small kinetic isotope effect observed when B—H is replaced by B—D, $\frac{k_{B-H}}{k_{B-D}} = 1{\cdot}52$, as being due to a non linear transition state in which the B—H stretching frequency is only partly lost in the transition state. When D_2O is used as reagent in the above reaction a much larger isotope effect, $\frac{k_{H_2O}}{k_{D_2O}} = 6{\cdot}90$, results indicating loss of most of the O–H stretching vibration in the water molecule involved in the transition state.

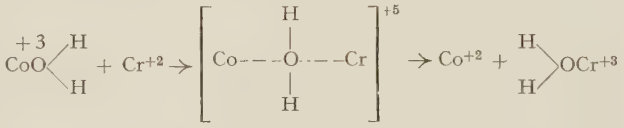


Hawthorne and Lewis point out that the most reasonable point of attack of an electrophile on a bond to hydrogen will be at the point of high electron density, that is, the bond itself[56,57]. A non linear transition state as above with consequent partial retention of the hydrogen stretching vibration in the transition state is not unexpected.

Cl. HO. etc. Transfer to Reductant. Taube *et al.* utilizing the fact that complex ions of Cr (III) are stable in aqueous solution, i.e., do not exchange their coordinated groups, have shown that atom transfer accompanies the oxidation of Cr (II) by certain oxidizing agents. Thus the oxidation of chromous ion by ferric ion which is catalyzed by chloride ions proceeds with transfer of coordinated chlorine from iron to chromium[58]; the activated complex being:

$$[Cr — Cl — Fe]^{+4}$$

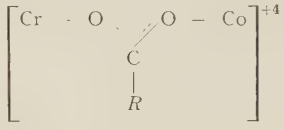
Similarly the chlorine atoms of $CoCl^{++}$, $AuCl_4^-$, and $Co(NH_3)_5Cl^{++}$ become incorporated in the coordination sphere of the Cr (III) formed when these reagents oxidize Cr (II). Indeed the oxidation of Cr (II) by Co (III) $(NH_3)_5X$ where $X = N_3^-$, Br^-, CNS^-, $CH_3CO_2^-$, PO_4^-, and SO_4^-, results in the transfer of X in every case[59]. Since these ligands are joined to both oxidizing and reducing agent in the activated complex they are called bridging groups. Hydroxyl ion and probably water can also serve as a bridging group in this reaction and on examining the oxygen—18 kinetic isotope effect when these groups are transferred, it is clear that the cobalt-oxygen bond is partially broken in the activated complex[60].



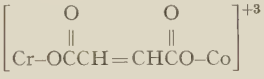
An interesting aspect of the Cr(II)–Co(III) oxidation is that if the bridging group is fumarate ion the rate is con-

	rate
$HO_2CCH=CHCO_2^-$	1·13
$CH_3CO_2^-$	0·15
$HO_2C–CH_2CH_2–CO_2^-$	0·19

siderably faster than if it is either acetate or succinate[61]. Presumably the carboxylate groups in acetate, succinate (and isophthalate) function as bridges in the activated complex by bonding to both cobalt and chromium as follows:



With fumarate ion (or terephthalate ion), a bridge of much lower energy can be formed using both carboxylate groups as follows:



The chromium and cobalt are now connected electronically through the π electron system of the bridging group allowing immediate electron reorganization to occur. Succinate ion can bond to both chromium and cobalt just as fumarate ion can but the bridging is ineffective because of the lack of electronic coupling.

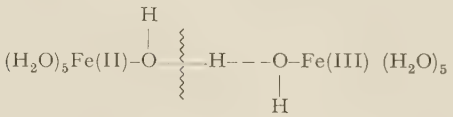
[55] J. Halpern, Quart. Revs. *10*, 463 (1956).
[56] M. F. Hawthorne and E. S. Lewis, J. Amer. chem. Soc. *80*, 2496 (1958).
[57] E. S. Lewis and M. C. R. Symons, Quart. Rev. *12*, 230 (1958).
[58] H. Taube and H. Myers, J. Amer. chem. Soc. *76*, 2103 (1954).
[59] H. Taube, J. Amer. chem. Soc. *77*, 4481 (1955).
[60] R. K. Murmann, H. Taube, and F. A. Posey, J. Amer. chem. Soc. *79*, 262 (1957).
[61] H. Taube, Canad. J. Chem. *37*, 129 (1959).

Since chromium undergoes a unit valence change in the above reactions it is apparent that the transferred group is effectively a radical. Now presumably these radical reactions proceed with homolytic bond rupture whereas the oxidations in which an oxygen atom or a hydride ion are transferred proceed with heterolytic bond rupture. Yet both kinds of reaction proceed in aqueous solution and do not show marked differences in character. There is not, in fact, the sharp dichotomy to which we have become accustomed in organic chemistry between radical reactions (homolytic bond cleavage) and the so-called ionic reactions (heterolytic bond cleavage)[62]. The former reactions, because of the production of neutral reactive radicals, often proceed by chain mechanisms and the rates are often largely independent of solvent whereas the inorganic species, like Cr (III) which are produced by radical transfer are stable ions. In this connection it is interesting that Halpern has shown that the homogeneous reaction of hydrogen with Ag^+ proceeds by two paths probably involving homolytic and heterolytic bond splitting respectively[55].

$$2\,Ag^+ + H_2 \rightarrow [Ag^+ - - - H - - - H \; - - Ag^+] \rightarrow 2\,AgH^+$$
$$Ag^+ + H_2 \rightarrow [Ag^{\delta+} - - - H - - - H^{\delta+}] \rightarrow AgH + H^+$$

Hydrogen Atom Transfer to Oxidant.—The reaction of silver ion with hydrogen just referred to is an example of this process. In addition there are many well substantiated examples of oxidations proceeding by way of hydrogen atom transfer from organic compounds to free radicals[63].

Hudis and Dodson have suggested that hydrogen atom transfer may be involved in the exchange between Fe^{+2} and Fe^{+3} in aqueous solution[64]. One might have expected some similarity to the $Fe(CN)_6^{-3}$–$Fe(CN)_6^{-4}$ exchange which proceeds by way of an electron jump through the firmly bound but polarizible coordination spheres of the central ions. However the exchange between Fe^{+2} and Fe^{+3} proceeds at only one half the rate in D_2O as in H_2O. The viscosity difference would account for only 20–30% of this effect and there seems to be little doubt that the solvent molecules play an important part in the reaction. Hydrogen atom transfer from hydrated ferrous ion, Fe(II) $(H_2O)_6$, to hydrolyzed ferric ion, Fe(III), $(H_2O)_5OH$ would account for these results, these two ions being involved, in the light of the kinetics, in the major reaction path[64].



The required zero free energy change is accomodated by this mechanism since reactants and products are identical. However the isotope effect may be due, not to hydrogen-oxygen bond breaking in the activated complex, but rather to a large solvent effect. It has been shown[65] that an isotope effect of some magnitude exists even for the reaction of $Cr(NH_3)_5Cl^{+2}$ with Cr (II), a reaction which was shown to take place by chlorine atom transfer[59]. It appears that more information on the solvating characteristics of H_2O and D_2O is required before the solvent isotope effect can be safely applied to the reactions of ions in solution.

Conclusions

The purpose of this article has not been to discredit in any way the commonly used definitions of oxidation in terms of electron transfer. Nor is it by any means intended to imply that oxidations do not occur in some cases by what could be called an electron transfer process. However sufficient evidence exists, some of which has been cited herein, to show that the mechanisms of a great many other oxidation reactions would be grossly oversimplified if they were considered as electron transfer processes. Most of these reactions are, in fact, represented quite satisfactorily by the familiar mechanisms of modern organic chemistry.

Acknowledgment.—The author is grateful to Drs. J. Halpern and K. B. Wiberg for helpful discussions.

Résumé

L'emploi des isotopes a prouvé que beaucoup d'oxydations procèdent d'échanges d'atomes ou de groupes d'atomes de l'oxydant au réducteur ou vice versa. Ici, le mécanisme des oxydations comportant l'échange de telles matières en tant qu'atomes d'oxygène, ions hydrides, atomes d'hydrogène et atomes de chlor sont étudiées et discutées.

CONGRESSUS

DENMARK

The Finson Memorial Congress

Copenhagen, July 31 to August 5, 1960

The 3rd International Congress on Photobiology will be held in Copenhagen July 31–August 5, 1960. President of the Finson Memorial Congress is Børge Chr. Christensen, M.D., Dr. Sc., The Finson Memorial Hospital, Strand boulevard, Copenhagen. The Secretary General of the Congress is Dr. techn. B. Buchmann, Biofysisk Laboratorium, Juliane Mariesvej 30, Copenhagen. The program of the Congress will consist of contributed papers and of a series of symposia on the following topics:

Biological action spectra;
Initial mechanisms involved in radiation effects;
Phototherapy;
Photoreceptors in aquatic organisms;
The results of the 3rd International Geophysical Year in regard to radiation;
Plant Cell response to visible light excluding photosynthesis;
Biological clocks;
The effects of long visible and near infrared radiation;
Photoreactivation–invited papers for a session.

Inquiries may be sent to the President or to the Secretary General of the Congress.

[62] C. K. Ingold, *Structure and Mechanism in Organic Chemistry* (Cornell University Press, Ithaca, N.Y. 1953), p. 205.

[63] W. A. Waters in *Organic Chemistry*, Vol. IV (H. Gilman, Ed., John Wiley and Sons, New York 1953), p. 1120.

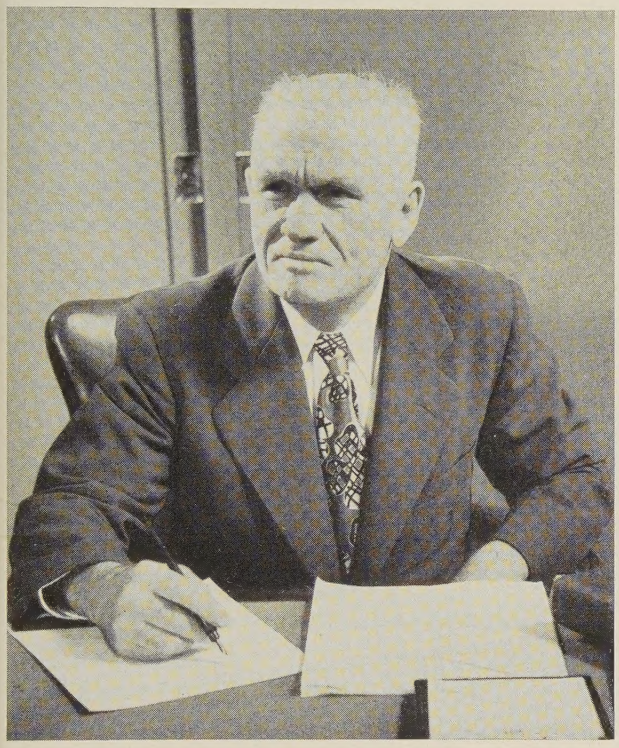
[64] J. Hudis and R. W. Dodson, J. Amer. chem. Soc. *78*, 911 (1956).

[65] A. E. Ogard and H. Taube, J. Amer. chem. Soc. *80*, 1084 (1958).

IN MEMORIAM

Carl G. Hartman's Contributions to the Physiology of Reproduction*

Few people know that CARL HARTMAN was immortalized when two species of fungus growing ants were named after him: *Atta Hartmanni Wheeler*, and *Trachymyrmex turrifex Wheeler caroli* Sub. sp., Wheeler. He is best known, however, for his basic contributions to the embryology of the opossum (Didelphis virginiana) and the reproductive physiology of the monkey (*Macacus rhesus*).



In 1912 Professor J. T. PATTERSON offered HARTMAN an instructorship in his department of zoology with the request to study the embryology of the opossum. Why the opossum? 'For the same reason that PATTERSON selected the armadillo: the prairie and the creek bottoms were full of both species and nobody had yet bothered to study the development of these, the most bizarre of all American mammals. It was, therefore, at the University of Texas that the essential characteristics of their mode of reproduction was cleared up.' (HARTMAN.) For thirteen years, he relates in a biographic sketch, 'I hunted the animals in the field. I also took care of them (at first at my home), operated on them, secured, fixed, sectioned, and stained their eggs and photographed the sections myself.'

Based on a thorough study of nearly 1000 females from which several thousand eggs were obtained, HARTMAN established the important stages in the intra- and extra-uterine development of the opossum. He found that the opossum is polyestrous with a modal length of the cycle between 28 and 30 days. During its tubal journey, the ovum of the opossum first acquires a heavy albuminous coat, and later, in the distal parts of the tubes, a soft shell. He tagged ova at ovulation with Ascaris eggs and recovered them 25 h later in the uterine folds. He thus proved that the ovocyte of the opossum travels through the oviducts at a higher speed than do the eggs of the mouse, rat, rabbit, or pig. From these experiments HARTMAN generalized that there must be a close and critical time relationship between ovulation and fertilization in mammals. Incidently, HARTMAN brought to the attention of the gynecological world the experiments of L. L. LEWIS, published in 1911, on timed matings in the sow, which supported a limited viability of the mammalian ovum. HAMMOND has later supplemented precise data for the short period of viability of the ovocyte in the rabbit. Together with the facts learned from the primates the notion of a restricted viability of the opossum egg has given great momentum to the research of the fertile and sterile days in the menstrual cycle of women.

The opossum was the first wild species to yield the complete story of its embryology and reproductive physiology. Most females were caught while already pregnant and, fortunately, mating dates were not known. To establish the chronology of successive stages in fetal development HARTMAN modified Bischoff's technique of timed and repeated uterotomies with fetal evacuations in the course of the same pregnancy. He discovered that, in the opossum, in each estrous cycle many more eggs are ovulated than ever become fertilized. In addition, 10 to 20% of the ovocytes fertilized degenerate at different stages during pregnancy. From observed matings and from timed operations on the pregnant opossum he learned something about the large degree of variability in the course of fetal development. Dating mammalian embryos as to their 'coital age' or as to specified 'days of pregnancy' is possible only with allowance for a wide margin of variability. This important fact has too often been neglected, even by present workers in the field.

HARTMAN established for the opossum what J. P. HILL had discovered in the kangaroo: at the time of parturition a temporary direct canal forms between the uteri and the distal vagina. The lateral vaginal canals participate in the changes of the estrous cycle. They, probably, serve as organs for semen activation and they facilitate the rapid transport of the sperm cells into the proximal parts of the fallopian tubes.

The span of gestation in the opossum lasts 12 to 13 days. At birth the newborn opossum weighs approximately 0·13 g. This tiny baby, by the force of his forepaws, crawls from the urogenital sinus into the maternal pouch and, for the next two months, affixes himself at one of the 13 teats. From his report the reader will sense something of the fascination HARTMAN experienced when he and his wife first witnessed the birth of the opossum (February 6, 1920).

By unilateral oophorectomy at specified intervals after mating HARTMAN demonstrated that one ovary alone suffices for the maintenance of pregnancy in both uteri. Bilateral oophorectomy interrupts pregnancy at any stage. Castration does not interfer with lactation.

When HARTMAN began to study the opossum, very little was known about this 'most stupid animal' (HARTMAN). The only monograph of some importance was E. SELENKA's *Studien über die Entwicklungsgeschichte der Thiere*, Vol. 4, *Das Opossum*. Thirteen years later, HARTMAN had described the main features of the anatomy of the sex organs, the estrus, ovulation and fertilization, the early embryology, the birth and postnatal development in the pouch of the opossum. His findings have been verified in-

* Paper read at his eightieth birthday, June 3, 1959.

dependently – and supplemented in a few details – by the work of E. McCrady (1938).

After he was through with the opossum Hartman felt ready for similar studies in the reproduction of primates. In 1925 he, therefore, planned an expedition to the philipine islands to gather monkey embryos. But G. L. Streeter of the Department of Embryology of the Carnegie Institution of Washington invited him 'to join his laboratory and study the monkey embryology the safe and sane way, bringing the monkey to the laboratory' (Hartman). G. W. Corner, in continuation of his investigations on the corpus luteum of the sow, had already begun to study the menstrual cycle in a few rhesus monkeys. In 1923 he demonstrated the first living primate egg. In Baltimore, then, Hartman took over Corner's monkey cages. He 'got acquainted with the monkey' and succeeded in raising and maintaining a large rhesus colony. Thus, for the first time, a primate became a laboratory animal.

By daily rectal palpations of the monkey ovaries Hartman estimated the time of ovulation and its relationship to the phases of the menstrual cycle. Through timed matings he produced the embryological material on which G. L. Streeter and Ch. H. Heuser based their *Developmental Horizons*, the first complete embryological series in a primate. With G. W. Corner, Hartman worked out the histogenesis of the corpus luteum. With G. B. Wislocki he indulged in the development of the primate placenta. With Ines de Allende he studied the cytology of the vagina in the course of the menstrual cycle. He provided timed specimens for G. W. Bartelmez' detailed study of the histology of the mucosa uteri.

Hartman showed that the frequency of anovulatory cycles explains the relative sterility during adolescence in the primates. The bleeding which frequently occurs at the time of implantation, in the monkey as well as in women, carries his name (Hartman's sign).

In 1932 he summarized his monkey work in the now classic monograph: *Studies in the Reproduction of the Monkey*. His general review *Time of Ovulation in Women* (1936) marks a milestone in the search for facts in human reproduction.

Hartman holds a central position among the workers on reproductive physiology during the first half of the twentieth century. His broad approach to the special subject and his inexhaustible industry secured lasting results. Many workers in the field owe to him research suggestions, material help, and friendly advice or sound criticism. He contends that he never said to himself: 'I am going to be a scientist... I never bothered to consider: is there any use in this study? The main thing was that I was having fun doing the experiments.' This, the essayist feels, is the secret why his 'researches were to occupy his waking hours to this good day ...'.

R. F. Vollman

Department of Anatomy, University of Illinois, Chicago, March 16, 1959.

Zusammenfassung

Der Bericht schildert die Entwicklung des Philologiestudenten Carl G. Hartman zum Naturwissenschaftler. Studien über pilzzüchtende Ameisen und über die Ökologie solitärer Wespen folgten embryologische Versuche bei Fledermäusen. Mit hier anschliessenden Untersuchungen zur Fortpflanzungsphysiologie und frühen Embryonalperiode des Opossums und des Rhesusaffen hat Hartman einen grundlegenden Beitrag zur modernen Fortpflanzungsphysiologie geleistet.

Selected Bibliography of Carl G. Hartman's 253 Publications

'Anybody who is industrious can get results in research. Only once in a while do we have a basic idea that can be stated in a few pages ...' (Hartman).

Studies in the Development of the Opossum, Didelphis virginiana L.

I. *History of the Early Cleavage*, II. *Formation of the Blastocyst* (Doctor's Thesis), J. Morphol. *27*, 1 (1916).

III. *Description of New Material on Maturation, Cleavage, and Entoderm Formation.*

IV. *The Bilaminar Blastocyst*, J. Morphol. *32*, 1 (1919).

V. *The Phenomena of Parturition*, Anat. Rec. *19*, 251 (1920).

The Oestrous Cycle in the Opossum, Amer. J. Anat. *32*, 353 (1923).

Observations on the Viability of the Mammalian Ovum, Amer. J. Obstet. Gyn. *7*, 1 (1924).

Description of Parturition in the Monkey Pithecus Rhesus, together with Data on the Gestation Period and other Phenomena Incident to Pregnancy and Labor, Bull. Johns Hopkins Hosp. *43*, 33 (1928).

The Period of Gestation in the Monkey, Macacus Rhesus, first Description of Parturition in Monkeys, Size and Behaviour of the Young, J. Mammal. *9*, 181 (1928).

The Breeding Season of the Opossum and the Rate of Intra-uterine and Postnatal Development (Didelphis virginiana), J. Morphol. Physiol. *46*, 143 (1928).

The Fertile Period of the Menstrual Cycle in the Monkey, Anat. Rec. *44*, 226 (1929) (Abstr.).

On the Almost Instantaneous Transport of Spermatozoa Through the Cervix and Uterus in Rat, Proc. Soc. exp. Biol. Med., N. Y. *28*, 312 (1930) (with J. Ball).

Bimanual Rectal Palpation as Applied to the Female Rhesus Monkey, Anat. Rec. *45*, 263 (1930) (Abstr.).

On the Relative Sterility of the Adolescent Organism, Science *74*, 226 (1931).

Ovulation and the Transport and Viability of the Ovum and Sperm in the Female Genital Tract, in E. Allen's *Sex and Internal Secretions* (Hoeber 1932), p. 647, second Ed. 1939, p. 630.

Studies in the Reproduction of the Monkey, Macacus Rhesus with special Reference to Menstruation and Pregnancy, Contrib. to Embryol. Carnegie Inst. of Wash. *23*, 1 (1932).

Pelvic (rectal) Palpation of the Female Monkey, with Special Reference to the Ascertainment of Ovulation Time, Amer. J. Obstet. Gyn. *26*, 600 (1933).

Anatomy of the Rhesus Monkey (Williams & Wilkins 1933).

Time of Ovulation in Women (Williams & Wilkins 1936).

Recovery of Primate Eggs and Embryos; Methods and Data on the Time of Ovulation, West. J. Surg. Obstet. Gyn. *52*, 41 (1944).

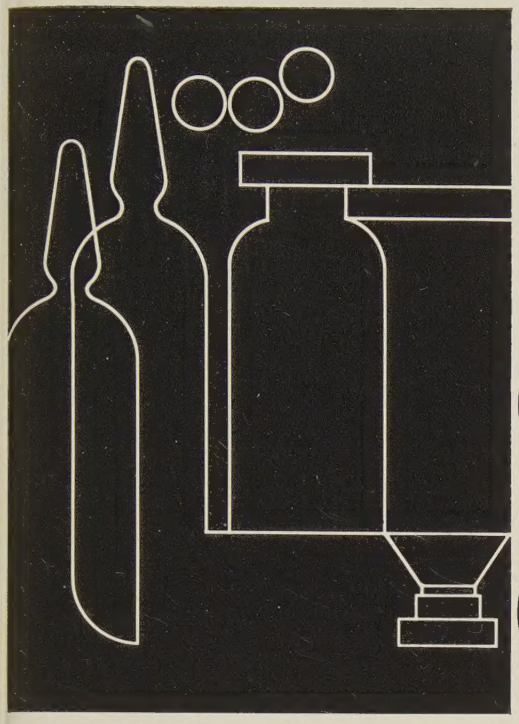
The Mating of Animals (Mammals) in Animal Colony Maintenance, An. N. Y. Acad. Sci. *46*, 23 (1945).

Possums (Texas University Press 1952).

How Variable is the Functional Life of the Human Corpus Luteum Menstrualis (Ogino Festschrift), World of Obstet. Gyn. *7*, 39 (1955).

How do Sperms get into the Uterus? Fertility and Sterility *8*, 403 (1957).

Uterotubal Insufflation, Obstet. Gyn. *11*, 622 (1958).



Die pharmazeutischen

Spezialitäten SANDOZ

geniessen

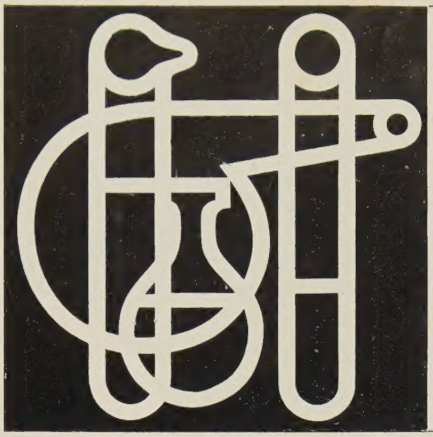
das Vertrauen

der Ärzte

in der ganzen Welt

SANDOZ A.G., Basel

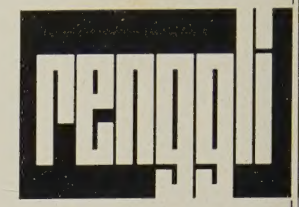
SANDOZ



Laboratorien

ILMAC Halle 5 Stand 587

Wir liefern Einrichtungen für chemische und biologische Laboratorien sowie für Labors physikalischer, analytischer, bakteriologischer und pharmazeutischer Zweckbestimmung. Säurefeste Tisch- und Wandbeläge. Armaturen für Gas, Wasser und Druckluft. Apparate und Geräte. Fragen Sie uns bitte an.



E. Renggli Zug Poststr 14 Fabrik für Laboratorien Tel 042 4 02 26

V. B. WIGGLESWORTH

Professor an der Universität Cambridge (England)

PHYSIOLOGIE DER INSEKTEN

Zweite unveränderte Auflage 1959

Übersetzung aus dem Englischen von MARTIN LÜSCHER, Professor an der Universität Bern. 832 Seiten mit 355 Abbildungen. In Ganzleinen Fr./DM 32.–. Band 14 der Reihe der experimentellen Biologie, Sammlung «Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften»

Inhalt: Vorwort. 1. Die Entwicklung im Ei. – 2. Das Integument – 3. Das Wachstum – 4. Muskelsystem und Fortbewegung – 5. Das Nervensystem – 6. Sinnesorgane – 7. Sinnesorgane: Mechanische und chemische Sinne – 8. Das Verhalten – 9. Die Atmung – 10. Das Zirkulationssystem und damit in Verbindung stehende Gewebe – 11. Verdauung und Ernährung – 12. Exkretion – 13. Der Stoffwechsel – 14. Wasser und Temperatur – 15. Fortpflanzung – Autorenverzeichnis – Sachverzeichnis

«Das Buch gehört auf den Arbeitstisch jedes Zoologen und Entomologen. Nicht zuletzt findet aber der in der angewandten Entomologie und im Pflanzenschutz Tätige und gerade er hier die Grundlagen eines Wissens vereinigt – eines Wissens, das er zur tiefern Durchdringung und Erfassung seiner praktischen Probleme dringend benötigt.» *Pflanzenschutz*

Zu beziehen durch Ihre Buchhandlung – Obtainable from your bookseller – Commandes à votre libraire

BIRKHÄUSER VERLAG · BASEL UND STUTTGART